RECURRENT INOCULATION OF SOYBEANS IS NOT NECESSARY

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Abstract. *Soybean is a legume used world-wide for food and fodder. It fixes atmospheric nitrogen and symbiotically produces high quality protein, and is therefore a valuable crop to include in any crop rotation. Currently Sweden imports large quantities of soybean, notwithstanding the negative impact on the environment, owing to, e.g., deforestation in countries where soybeans are produced and the use of long transportation routes. When cultivating soybeans on juvenile soils, which are common in Sweden, they need to be inoculated with their symbiotic partner, the bacteria Bradyrhizobium japonicum. Task of the present study was to investigate the need for re-inoculation of soybeans in cropping sequences where soybeans are included. For this purpose we collected samples from soils in which soybeans had been grown previously and used these soils to grow soybeans in a greenhouse. We found that B. japonicum survived in the soils in which soybeans had been grown previously. Soil factors such as pH, nitrogen content and organic matter showed varying and inconsistent effects on the survival rate of B. japonicum, making it difficult to estimate their impact significance. However, application of nitrogen (as manure) and high amounts of phosphorus in soil appeared to promote B. japonicum survival. The amount of potassium in soil had varying effects, but high amounts appeared to impair B. japonicum survival. As regards to soil type, B. japonicum survived better in light soils than in clayey soils. We conclude that it could be recommended for growers not to re-incoulate their soybeans if the crop has been cultivated in recent years.*

Key words: *cropping sequences, Bradyrhizobium japonicum, re-inoculation, sustainable production.*

INTRODUCTION

Soybean has the ability to fix atmospheric nitrogen for producing high quantity and quality protein. This feature makes the crop suitable as a food and fodder; it is also a welcome feature in the cropping sequence [1]. In Sweden, soybean is not cultivated to a great extent, therefore Sweden relies on import. In 2011, soybean was the second largest food import in Sweden, comprising about 250 thousand tons valued at approx. 125 million USD, aimed primarily as animal fodder. Importing soybean has a negative impact on the environment through, e.g., deforestation and long transportation routes before arriving in Sweden. To counteract this, the interest in growing soybean in Sweden has increased. To ensure that an effective nitrogen-fixing symbiosis establishes, farmers need to inoculate soybean seeds with *Bradyrhizobium japonicum* before sowing. Inoculation is simply done by applying *B. japonicum* directly onto the seeds. It is currently considered necessary to re-inoculate soybean seeds with *B. japonicum* each time the crop is to be grown to ensure the symbiotic nitrogen fixation to be established.This raises the question amongst growers whether annual inoculation is really necessary. A report has shown that *B. japonicum* bacteria are unable to survive in prairie soils in Canada [2]. Given this background, the aim of the presented work was to investigate if *B. japonicum* is able to survive in the soils of Sweden after a soybean crop, in order to give advice to growers regarding the necessity to re-inoculate soybeans in a cropping sequence including soybeans.

MATERIALS AND METHODS

Soil sampling and field management history

Soil samples were collected in 2014 from the following farms:

- Munktorp (N 59°32.301’, E 16°8.604c), sampling conducted from fields where soybean had been grown during 2011 and 2013;
- Sjöö (N 59°42.616’, E 17°30.293’), sampling conducted from three fields where soybean had been grown in 2011, 2012 and 2013;

– Edsberg (N 59°22.391’, E 13°15.022’), sampling conducted from fields where a soybean crop had been grown in the previous year (i.e. 2013);
– Berga (N 59°11.433’, E 14°52.910’), sampling conducted from fields where a soybean crop had been grown in the previous year (i.e. 2013).

Soil samples were taken from the topsoil (0-20 cm depth). A total of 20-30 samples of 1-2 L soil, each consisting of 5-6 subsamples, were collected randomly diagonally at each field. Immediately after collecting the soil, samples were mixed with pumice stone in order to avoid compact soils, and placed in pots comprising 1.3-1.4 L soil and 0.6-0.7 L pumice stone. Five soybean seeds, cv. Moravians, were then placed in each pot. Conditions set for the soybeans were 20^{o}C, light intensity of 250-350 µmol m^{-2} s^{-1}, duration of light 17 hours/day and humidity 60%. No additional nutrients were applied to the plants during growth. After seedling emergence, plants were thinned to give two plants per pot. Manual watering was performed when necessary. Plants were harvested after two months of growth. Above ground plant material and nodules were separated, dried and weighed. The soil from each field was analysed for pH value and content of nitrogen, phosphorus, potassium and carbon using standard procedures.

Statistical analysis

For each experiment mean values of each treatment were calculated and compared with each other using the two-sample t-test, using Minitab program [3].

RESULTS AND DISCUSSION

All collected soil samples contained *B. japonicum*, since all soybean plants produced nodules (Table 1).

Table 1

Symbiotic efficiency of *Bradyrhizobium japonicum* in soils where soybeans were cultivated the previous year (2013)

Location	Nodule weight (g)	Plant biomass weight (g)	Nitrogen content in plant biomass (%)
Berga	0.42 ± 0.04C	15.0 ± 1.10B C?	3.12 ± 0.16D
Edsberg	0.40 ± 0.02C	13.6 ± 0.62B C?	2.87 ± 0.12C
Munktorp	0.26 ± 0.01B	10.8 ± 1.20B	2.27 ± 0.06B
Sjöö	0.14 ± 0.01A	6.48 ± 0.84A	2.55 ± 0.08A

Different letters indicate that the mean values (n=7) are significantly different.

The soil at Munktorp, in which soybean had been grown previously in 2011 and 2013, still contained *B. japonicum*, since all plants in each treatment produced nodules. There were no differences between years in terms of nodule weight or plant biomass weight. This indicates that there was a similar amount of surviving *B. japonicum* in the soils independently of for how long ago soybeans had appeared on the site (Table 2). As shown, nitrogen content in plants were higher in plants forming nodules with bacteria which has been able to survive for at least some years after the introduction.

Table 2

Symbiotic efficiency of *Bradyrhizobium japonicum* in soils at Munktorp where soybeans were cultivated in 2011 and 2013 respectively

Year of last soybean cultivation	Nodule weight (g)	Plant biomass weight (g)	Nitrogen content in plant biomass (%)
2011	0.24 ± 0.02	7.46 ± 0.51	3.41^{*} ± 0.08
2013	0.19 ± 0.03	8.65 ± 0.95	3.06 ± 0.16

Asterisk (*) indicates significant differences between mean values (n=7).

Similarly, soils from Sjöö with soybeans grown previously in 2011, 2012 and 2013 all contained *B. japonicum*, as plants grown in these soils were able to produce nodules. There were no differences between the soils in terms of nodule weights. However, there were differences regarding to plant biomass weight and nitrogen content (Table 3). The lowest biomass was obtained in soil with soybeans grown in 2013, and the highest nitrogen content was achieved in plants grown in a soil cultivated with soybeans a year before.

Table 3

Symbiotic efficiency of *Bradyrhizobium japonicum* in soils at Sjöö where soybeans were cultivated in 2011, 2012 and 2013 respectively

Year of last soybean cultivation	Nodule weight (g) ± SE	Plant biomass weight (g) ± SE	Nitrogen content in plant biomass (%)± SE
2011	0.53 ± 0.05	15.1 ± 1.00	2.71 ± 0.12
2012	0.34 ± 0.03	11.4 ± 1.10	3.01* ± 0.10
2013	0.19 ± 0.02	8.60* ± 0.54	2.81 ± 0.17

Asterisk (*) indicates significant differences between mean values (n=7).

As presented, *B. japonicum* were present in all soils, since the plants in all soils produced nodules (Tables 1-3). As shown, the bacteria were able to survive in soil after two years, which contradicts with previous study [2] where it was found that *B. japonicum* was unable to survive in Canadian soils. The reason of the discrepancy could be due to current soil characteristics. Soil characteristics, such as pH, have been shown to affect *B. japonicum*. The bacteria have been shown to prefer higher pH [4]. In our study a trend was found between soil pH at the sites and biomass, and nodule weights (Figure 1).

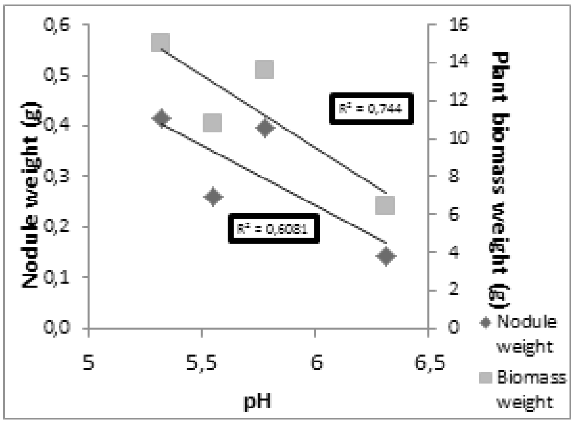

Figure 1. **The effect of soil pH on nodule dry weight and plant dry biomass of soybean grown in soils where soybean had been grown a year before previously (2013)**

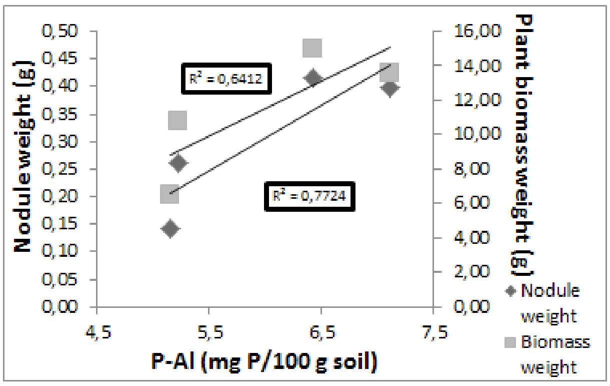

Figure 2. **Effect of plant available phosphorus (P-AL) on nodule dry weight and plant dry biomass of soybean grown in soils where soybean had been grown a year before previously (2013)**

There were no correlations between nodule weight and plant biomass weight, and soil nitrogen and carbon content (data not shown). This observation could not be confirmed since there are no previous reports available on of how soil organic matter and nitrogen in soil affect *B. japonicum*. Analysis on the relationship between plant available P (P-AL) phosphorus (P) and survival of *B. japonicum* indicates that plant available P may have an impact on the nodule weight and plant biomass (Figure 2). However, strongly bound soil P did not affect those parameters (data not shown). Our observations are supported by previous studies [5],[6], showing that higher soil phosphorus concentration promotes growth of *B. japonicum* and therefore soybean plants. Based on these results, we suggest that increased amount of phosphorus in soil increases the survival rate of *B. japonicum*.

Contrary to the beneficial effect of P on bacteria survival, potassium (K) negatively affects the survival of bradyrhizobia (Figure 3). Premaratne & Oertli [6] found that increased K concentration increased plant dry matter and nodule weight, which contradicts with our results. We further tested if the negative impact of K might be related to the soil texture, but this was not confirmed (data not shown). A better survival rate of *B. japonicum* has been observed in lighter soil rather than in loamy textured soils [7].

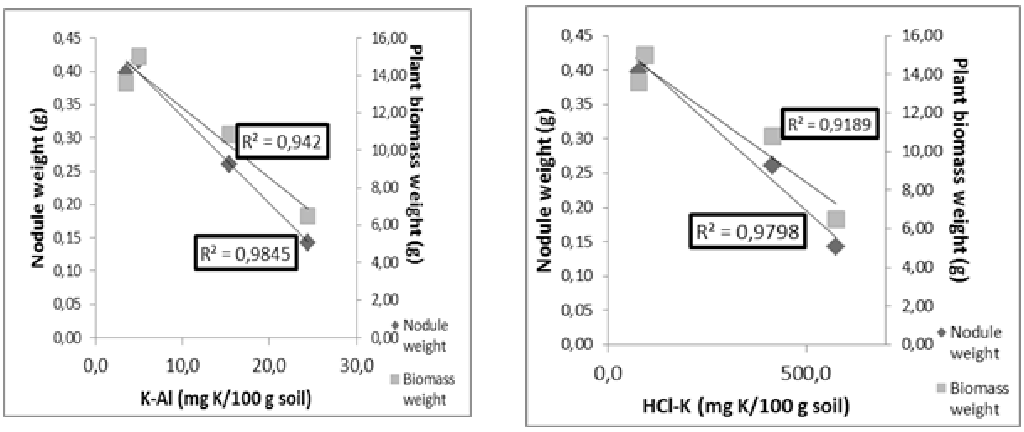

Figure 3. **Effect of plant available potassium (K-AL) (left) and strongly bound potassium (K-HCl) (right) on nodule dry weight and plant biomass of soybean grown in soils where soybean had been grown a year before previously (2013)**

CONCLUSIONS

B. japonicum is able to survive in the soils in which soybeans had been grown previously.

Soil factors, such as pH, nitrogen content and organic matter, have varying and inconsistent effects on the survival rate, making it difficult to estimate their impact.

Fertilization with phosphorus promotes *B. japonicum* survival.

High rates of potassium in soil appear to impair *B. japonicum* survival.

It could be recommended for growers not to re-inoculate their soybeans if the crop has been cultivated in recent years.

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