

Effect of combined fertilization with rock phosphate and elemental sulphur on yield and nutrient uptake of soybean

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ABSTRACT

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Greenhouse pot experiments were carried out in 2013 and 2014 at the University of Bonn, Germany, to study the effect of combined fertilization of rock phosphate (RP) with elemental sulphur (ES) on growth, grain yield and nutrient uptake of soybean. Treatments included RP, ES, combined application (RPES), triple superphosphate (SP), magnesium sulphate (MgS), SP and magnesium sulphate (SPMgS) and an unfertilized control. Combined fertilization (RPES) resulted in a significant increase of soil-plant analysis development (SPAD) values, grain yield, yield components and nitrogen and in part phosphorus uptake of the shoot. Single application of RP or ES only tended to affect crop growth and nutrient uptake. Application of MgS and/or SP significantly increased grain yield in both years suggesting an effect of sulphate.

Keywords: pot trials; organic farming; phosphorous deficiency; *Glycine max*

The EU-regulation 834/2009 only allows the application of rock phosphate (RP) as mineral phosphorus (P) fertilizer in organic farming. However, in most arable soils, unless very acidic, the solubility of RP is very low (Walker et al. 2006). Phosphorus deficiency can limit nodule formation, N₂ fixation (Carsky et al. 2001) and subsequent yield of grain legumes known to have a high P demand (Giller and Cadish 1995, Schulze et al. 2006). Former studies have shown that the combined application of RP with elemental sulphur (ES) can increase the P solubility of RP, which is assumed to be caused by sulphur-oxidizing bacteria such as *Thiobacillus* spp. producing sulphuric acid (Lipmann et al. 1916, Kittams and Attoe 1965, Schofield et al. 1981). Granulating RP with ES increased P availability (Rajan 1983, 1987) resulting in the development of bio-superphosphate featuring high efficacy on acid tropical soils (Stamford et al. 2003). The present study was conducted to assess the

effect of combined ES and RP application on growth, yield and nutrient uptake of soybean (*Glycine max* (L.) grown under greenhouse conditions.

MATERIAL AND METHODS

The experiments were conducted in Kick-Brauckmann pots (volume 10 L) in 2013 and 2014 in a greenhouse at the University of Bonn. In both years, crops were grown in a mixture of topsoil (sandy loam texture) from the research farm Dikopshof (50°49'N, 6°58'E) and sand (2:1). Soil chemical status (before mixing with sand) was 0.74% carbon (C), 0.06% nitrogen (N), 5.2 mg P and 11.4 mg potassium (K) per 100 g and a pH of 6.6 (see method description below).

Experimental design. The one-factorial experiment consisted of seven treatments with different

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types and mixtures of P and sulphur (S) fertilizers arranged in a randomized complete block design with four replications. Treatments included elemental sulphur with 95% S, magnesium sulphate (MgS), fine-ground rock phosphate with 11.4% P, either alone or combined with fine ground ES with 95% S (RPES), triple superphosphate (SP = $(\text{Ca}(\text{H}_2\text{PO}_4)_2 \times \text{H}_2\text{O})$), SPMgS and a zero-control (CON). Basal nutrient supply of all treatments is summarised in Table 1.

Pots were set up on a turnable framework on drays allowing both regular randomization and exposition to outdoor air on hot and dry days. Soybeans (*Glycine max* L.) cv. Sultana were sown (2.5 cm depth) in April with a density of nine seeds per pot and later adjusted to three plants. In 2013 seeds were inoculated with a liquid solution of *Bradyrhizobium japonicum* (Radicin®), which proved to be ineffective with respect to nodulation. For that reason all soils in the pots received an additional dressing with ammonium nitrate (0.6 g N per pot) split over four dates between 61 and 98 days after sowing. In 2014, seeds were successfully inoculated using a dry product based on peat with 4×10^9 viable bacteria per gram (HiStick®).

After planting the soil water content was adjusted to 50% of the maximum water holding capacity and later in the season to 65%. Soil water content was controlled daily to replenish water loss with deionized water. Plants were treated with Pyrethrins (Spruzit®)

against pea thrips (*Kakothrips pisivorus*) and the two spotted spider mite (*Tetranychus urticae*).

Data collection and assessments. The relative chlorophyll content (SPAD (soil-plant analysis development) value) of the three youngest, fully expanded leaves was weekly measured on each plant using a SPAD-Meter (SPAD-502, Konica Minolta, Osaka, Japan). The number of pods of all plants was counted shortly before harvest. At maturity in August, the whole plants were cut just above the soil surface. Shoots (pooled stems, leaves and empty pods) and grains of the soybean were analysed separately and total shoot nutrient uptake was calculated. Before determination of dry matter (DM) yield, grains were oven-dried until constant weight at 60°C and shoot biomass at 105°C. Grains were ground in a cryogenic mill (Spex CertiPrep 6750, Flex) and the shoot biomass in a vibratory disc mill (RS200, Retsch®).

Lab analyses. The soil pH was determined by a pH meter (Model WTW pH 340) with 10 g of soil + 60 mL of a 0.01 mol/L CaCl_2 solution. The pH meter was calibrated with standard buffer solutions of pH 4.0 and pH 7.0. Plant available K and P were measured after extraction with 0.1 mol/L calcium acetate + 0.1 mol/L calcium lactate + 0.3 mol/L acetic acid with a soil: extract ratio of 1:20 after shaking for 2 h (Schüller 1969). Ground grains and shoot biomass (200 mg) were digested with 3 mL conc. H_2O_2 and 6 mL conc. HNO_3 in a microwave oven. Hence P was measured with a photometer

Table 1. Fertilizer application at individual treatments

Nutrient	Fertilizer form and quantity per pot	Treatment						
		CON	ES	RP	RPES	MgS	SP	SPMgS
Nitrogen (3 g)	8.572 g NH_4NO_3	×	×	×	×	×	×	×
Phosphorus (0.2 g)	1.554 g of RP			×	×			
	1.0762 g $\text{Ca}(\text{H}_2\text{PO}_4)_2 \times \text{H}_2\text{O}$						×	×
Potassium (1 g)	1.906 g KCl	×	×	×	×	×	×	×
Magnesium (0.121g)	1.012 g $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$	×	×	×	×		×	
	1.227 g of MgSO_4					×		×
Sulphur (0.16g)	elemental sulphur		×		×			
	MgSO_4					×		×
Trace elements*		×	×		×	×	×	×

RP – soft ground rock phosphate 26 from the company Timac-Potasco. *0.483 g $\text{FeCl}_3 \times 6 \text{H}_2\text{O}$, 0.072 g $\text{MnCl} \times 4 \text{H}_2\text{O}$, 0.041 g ZnCl_2 , 0.056 g $\text{CuCl}_2 \times 2 \text{H}_2\text{O}$, 0.028 g H_3BO_3 , 0.012 g $\text{Na}_2\text{MoO}_4 \times \text{H}_2\text{O}$ and 0.024 g $\text{Co}(\text{NO}_3)_2 \times 6 \text{H}_2\text{O}$; CON – unfertilized control; ES – elemental sulphur; RP – rock phosphate; RPES – combined application RP and ES; MgS – magnesium sulphate; SP – triple superphosphate; SPMgS – combined application SP and MgS

(SAN plus System, Skalar, Breda, the Netherlands) and K with a flame photometer (Analyst 200 AAS, Perkin Elmer, Waltham, USA). Soil and plant C, N and S contents were determined with an elemental analyser (Eurovector, Milano, Italy).

Statistical analysis. Data sets were first analysed for normal distribution and homogeneity of variance using the Shapiro Wilk and the modified Levene test and then subjected to ANOVA followed by the Tukey's test ($\alpha = 0.05$). Since most evaluations showed significant interactions between treatment and year and due to the missing nodulation in 2013, the results are presented separately for each year.

RESULTS

SPAD-value. In 2013, the SPAD values showed a clear decrease from 44 to 58 days after sowing (DAS) before slightly increasing again up from 86 DAS (Figure 1). The significantly lowest SPAD values were always recorded in the control treatment, while significant differences between the treatments RP, RPES and SPMgS were not noted at 58 DAS. In 2014, the SPAD values tended to be rather stable between 51 DAS and 107 DAS with the exception of CON. Again, the significantly lowest SPAD values were recorded in CON as compared to all other treatments up from 86 DAS. From 72 DAS to 86 DAS the SPAD values of plants fertilized with SP tended to be higher than in the treatments RP and RPES. In contrast, at later growth stages (93–107 DAS) the highest SPAD values were recorded in the treatment MgS followed by RPES (Figure 1).

Biomass, grain yield and yield components.

Fertilizer application always resulted in a significant increase of shoot biomass as compared to the control (Table 2). In 2013, shoot biomass (without grains) ranged between 2.6 (CON) and being significantly highest in the treatment SPMgS with 7.9 g DM per plant.

In 2014, the shoot biomass was clearly higher ranging between 7.6 (CON) and 14.6 g DM per plant (SPMgS). The application of ES, MgS and RP only resulted in a slight increase of shoot biomass. In contrast, the combined application RPES or the application of SP and SPMgS gave significantly higher shoot biomass than CON (Table 2).

In analogy to the shoot, grain dry matter yield in 2013 was significantly higher in all fertilized treatments compared with CON. Likewise, the SPMgS treatment gave a significantly higher grain yield than all other treatments (Table 2). The average grain yield was four times as high (8.5 g per plant) in 2014 compared with 2013 (2.1 g per plant). In 2014 only the treatments MgS (8.1 g), RPES (8.6 g) as well as SP without (11.1 g) and with MgS (13.5 g per plant) gave significantly higher grain yields than CON. In contrary, in 2013 the harvest index of all treatments was significantly higher compared with the control, in 2014, a significantly higher HI was only noted in the treatment SPMgS. In 2014, the HI was clearly higher compared with 2013.

In both years, all fertilizer treatments (except RP and ES in 2014) resulted in a significant increase of pod setting with no further differentiation between the treatments in 2013. In 2014, the significantly highest number of pods was recorded in the SP treatments with 27.8 and 32.3 pods per plant in

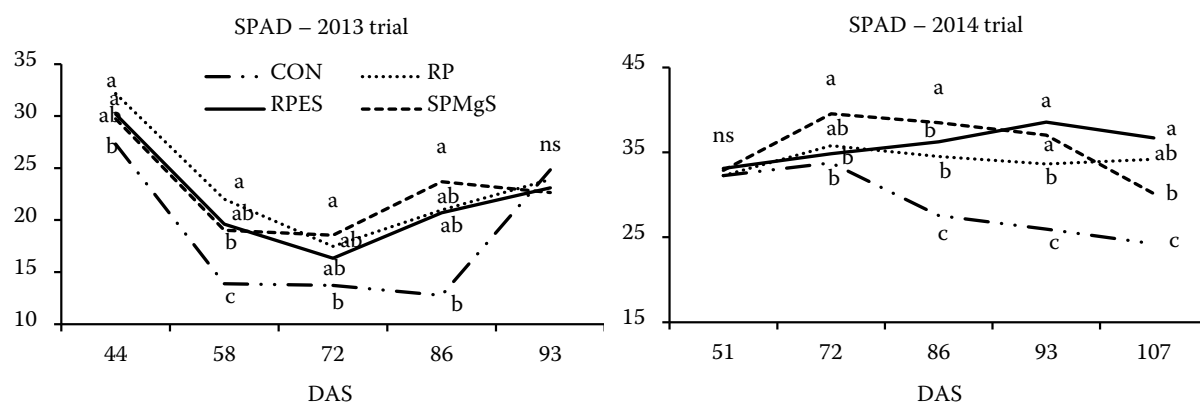


Figure 1. Effects of different treatments on soil-plant analysis development (SPAD) values of soybean leaves (2013 and 2014 trials). Means followed by different letters are statistically different ($P \leq 0.05$). DAS – day after sowing; CON – unfertilized control; RP – rock phosphate; RPES – combined application RP and ES (elemental sulphur); SPMgS – combined application SP and MgS

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Table 2. Effects of different treatments on yield components and yield in soybean at harvesting time in 2013 and 2014 trials

	ShDM (g/plant)		GDMY (g/plant)		HI		Pods/plant		Seeds/pod		TKW (g)	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
CON	2.6 ^c	7.6 ^c	0.6 ^c	4.5 ^d	0.18 ^b	0.37 ^b	4.4 ^b	12.5 ^c	1.6 ^b	2.3	87 ^b	160 ^b
ES	5.2 ^b	8.6 ^{bc}	2.1 ^b	6.0 ^{cd}	0.29 ^a	0.41 ^{ab}	11.0 ^a	15.3 ^{bc}	1.9 ^{ab}	2.3	104 ^{ab}	175 ^{ab}
MgS	4.7 ^b	10.1 ^{bc}	2.0 ^b	8.1 ^{bc}	0.30 ^a	0.45 ^{ab}	9.8 ^a	19.5 ^b	2.0 ^{ab}	2.3	105 ^{ab}	179 ^{ab}
RP	4.9 ^b	9.8 ^{bc}	2.0 ^b	6.7 ^{cd}	0.29 ^a	0.41 ^{ab}	9.7 ^a	16.9 ^{bc}	1.9 ^{ab}	2.3	110 ^{ab}	173 ^{ab}
RPES	5.2 ^b	10.8 ^b	2.0 ^b	8.6 ^{bc}	0.27 ^a	0.45 ^{ab}	10.2 ^a	21.1 ^b	1.9 ^{ab}	2.3	99 ^b	174 ^{ab}
SP	5.8 ^b	13.6 ^a	2.3 ^b	11.1 ^{ab}	0.28 ^a	0.44 ^{ab}	9.5 ^a	27.8 ^a	2.1 ^a	2.1	112 ^{ab}	182 ^a
SPMgS	7.9 ^a	14.6 ^a	3.5 ^a	13.5 ^a	0.30 ^a	0.48 ^a	12.4 ^a	32.3 ^a	2.2 ^a	2.2	129 ^a	193 ^a
Mean	5.2	10.7	2.1	8.4	0.27	0.43	9.7	20.8	1.9	2.2	106	177

Means followed by different letters are statistically different ($P \leq 0.05$). ShDM – shoot dry matter; GDMY – grain dry matter yield; HI – harvest index; TKW – thousand kernel weight; CON – unfertilized control; ES – elemental sulphur; RP – rock phosphate; RPES – combined application RP and ES; MgS – magnesium sulphate; SP – triple superphosphate; SPMgS – combined application SP and MgS

SP and SPMgS, respectively, followed by the treatments RPES and MgS with 21.1 and 19.5 pods per plant, respectively.

In 2013, the number of seeds per pod was significantly higher after application of SP and SPMgS, while all other treatments only tended to be higher than CON. In 2014, the number of seeds per pod was not affected by the treatments.

In both years, only the application of SP resulted in a significant increase of the thousand kernel weight, while the other treatments only tended to be higher than CON (Table 2).

Shoot biomass and grain nutrient content and total nutrient uptake. In 2013, the highest shoot and grain nutrient content (N, P, S) were noted in CON. In 2014, the SP treatments had a significant effect on the P content of the grain, while no effect was noted from RP and RPES. In contrast to elemental S, sulphate containing fertilizers (SPMgS, MgS) significantly increased the S content in both the shoot and grain biomass (data not shown). Variations in total biomass production and nutrient content resulted in differences of total nutrient uptake between the treatments.

The total nutrient uptake (shoot and grain) was higher in 2014 than in 2013 and significantly affected by the use of different fertilizers (Figure 2).

In 2013, the significantly highest total N uptake was noted after application of SPMgS followed by all other fertilizer treatments featuring significantly higher N uptake than CON. In 2014 the N uptake

of both SP treatments was highest, however not significantly different from the treatments MgS and RPES. The N uptake of the treatments ES, MgS and RP only tended to be higher than CON.

In both years, the total P uptake was lowest in CON. In 2013, P uptake of the treatment SPMgS was significantly higher compared with all other fertilizer treatments, which again significantly differed from CON.

In 2014, a significantly higher P uptake was only noted in the SP treatments, while P uptake of the other treatments only tended to be higher than CON. The S uptake in RPES was clearly higher than in CON however not significant. The S uptake in CON was the lowest in both years. In 2013, the treatments receiving sulphate (MgS and SPMgS) had a significantly higher S uptake than CON. In 2014, only the S uptake of the SP treatments was significantly higher than CON.

DISCUSSION

It is well established that limited amounts of plant available P in soils may result in a yield decrease and N_2 fixation of legumes may be impaired. However, in organic farming the application of water soluble P fertilizers like triple superphosphate is not allowed. For this reason, rock phosphate is the only alternative to apply mineral phosphorus. The disadvantage of rock phosphate is very low

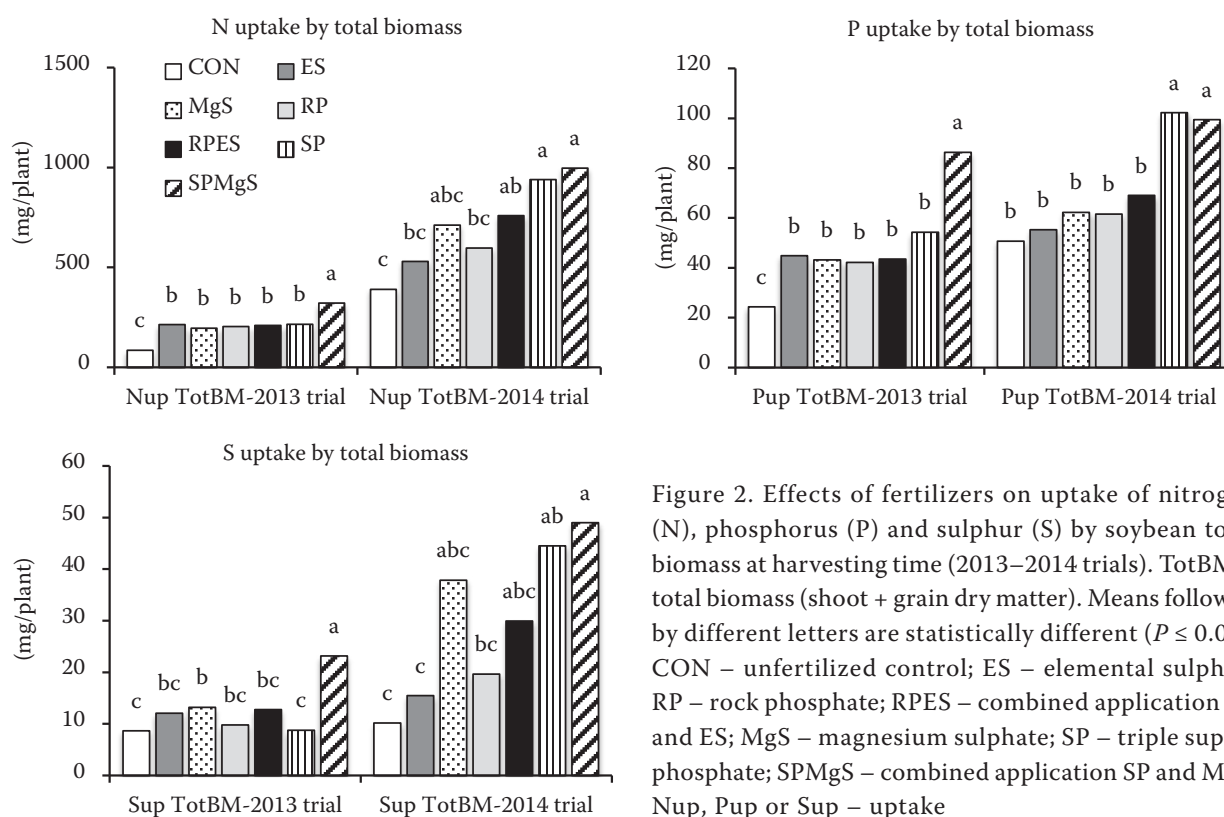


Figure 2. Effects of fertilizers on uptake of nitrogen (N), phosphorus (P) and sulphur (S) by soybean total biomass at harvesting time (2013–2014 trials). TotBM – total biomass (shoot + grain dry matter). Means followed by different letters are statistically different ($P \leq 0.05$). CON – unfertilized control; ES – elemental sulphur; RP – rock phosphate; RPES – combined application RP and ES; MgS – magnesium sulphate; SP – triple super-phosphate; SPMgS – combined application SP and MgS; Nup, Pup or Sup – uptake

solubility in the normal pH range of arable soils. For this reason, the combined application of rock phosphate and elemental sulphur was suggested to increase P-solubility (Rajan 1983, Evans et al. 2006, Walker et al. 2006). In our trials, the combined application of RP and ES resulted in a significant increase of crop growth parameters. The increased productivity is probably due to higher leaf SPAD values and nitrogen contents inducing a higher photosynthetic activity of soybeans (Monje and Bugbee 1992, Ma et al. 1995). The number of pods per plant was the only yield component affected by fertilization. This result is in line with the general assumption that the number of pods is the main factor for yield compensation and yield stability of soybean (Board and Tan 1995). In contrast, grain yields are typically not affected by the thousand-kernel weight and the number of seeds per pod (Schou et al. 1978, Herbert and Litchfield 1982). In the present trials, the combined application of RP and ES resulted in a significant increase of shoot biomass, grain yield and pods per plant compared with CON. Previous studies have also shown higher biomass production of wheat and rye grass after combined application of RP and ES on acidic soils (pH 4.3–5.4) in South-Australia

(Evans et al. 2006) and of rye grass in New Zealand (Rajan 1983). Likewise, yam bean yield in Brazil was significantly increased after RPES application, however only, if sulphur-oxidizing bacteria were amended (Stamford et al. 2003).

In contrast to biomass yield, the P and S uptake by the total biomass only showed a clear, but not significant trend for increase after RPES application. The missing statistical evidence is probably due to the high variation within the treatment replications.

Although the key abiotic factors including the particle size of RP and ES and soil moisture, that was constantly kept at 65 vol%, were favourable for sulphur oxidation (Attoe and Olsen 1966), the overall effect of RPES application was comparatively low in the present trials. Reasons for the weak, but in part significant effect of RPES, include a suboptimal, but still acceptable range of soil temperature of $\sim 20^{\circ}\text{C}$ (Janzen and Bettany 1987) and high soil pH of 6.6. It is also possible that the number and species of *Thiobacillus* present in the soil have limited sulphur oxidation.

The single application of either RP or ES only tended to increase soybean growth, P and S uptake. Phosphorus derived from rock phosphate

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is known to have a poor solubility (Dubey 1997, Steffens 2011). Likewise, elemental sulphur, a water-insoluble crystalline solid, can only be taken up by the plants after microbial oxidation.

In contrast, the treatments with triple superphosphate (SP and SPMgS) had the strongest impact on crop growth including SPAD values, shoot dry matter and grain yield indicating that soybean has a preference for water soluble P (Mustafa et al. 2004, Darwesh et al. 2013).

Application of sulphate (MgS) only tended to increase crop growth, while significantly increasing S uptake of the shoot and grain yield. The low S content of the grains in 2014 (0.24% on average) and the wide N:S ratio (28:1) suggest that insufficient S supply may have limited crop growth and yield except for treatments receiving sulphate. Both values were on average within the critical range (N:S < 22:1 and S% > 0.27%) suggested by Salvagiotti et al. (2012).

The highest P and S contents of shoot and grain were noted in the control and are a function of stunted growth causing a concentration effect. Inverse relationships between crop yield and mineral concentrations are widely known as a 'dilution effect' caused by fertilization, irrigation, and other management factors (Jarrell and Beverly 1981).

The poor soybean growth in 2013 is most probably due to missing nodulation, since visual assessments of roots after harvest showed no nodules at all. The induced nitrogen deficiency could easily be monitored by observing the SPAD values of the leaves (Figure 1), known to be strongly correlated with leaf nitrogen supply (Dwyer et al. 1995, Fontes and Araujo 2006) and by a lower harvest index (Hay and Gilbert 2001). While still at a normal level at 44 DAS, a clear decrease was noted at 58 DAS. Nitrogen application at 61 DAS resulted in a stabilization of SPAD values and even a slight increase. It is assumed that the missing infection of the roots with *Bradyrhizobium japonicum* resulting in nitrogen deficiency and low yields can be attributed to the poor quality of the strain used (Egamberdiyeva et al. 2004, Kumaga and Ofori 2004, Salvagiotti et al. 2008). Recent field trials in Germany with different inoculants for soybean including the one of our experiment in 2013 confirmed the insufficient quality of Radicin® (Wächter et al. 2013).

Our findings indicate that the combined application of rock phosphate and elemental sulphur has

the potential to improve the P nutrition of soybean. Effective utilization of this source, however, may require a higher control of abiotic factors (soil moisture, pH) and depends on biotic factors such as the number and species of sulphur-oxidizing bacteria. Under favourable environmental conditions, a combined application of fine ground RP and ES has the potential to improve nutrient supply and subsequently yield of soybeans in particular in organic farming. A promising biological approach could be to mix fine ground rock phosphate with elemental sulphur and subsequent inoculation with *Thiobacillus* spp.

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