Crop yields, boron availability and uptake in relation to phosphorus supply in a field experiment

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ABSTRACT

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The boron (B) availability and uptake were studied in relation to different phosphorus rates applied into soils in a three-year field experiment (2015–2017). The experiment was carried out at the experimental station at Humpolec (Bohemian-Moravian Highlands, Czech Republic). Three rates of phosphorus (20-40-80 kg P/ha) were applied as triple superphosphate. The crop rotation was spring barley-winter oilseed rape-winter wheat. No systematic fertilization with B was used and the response of natural boron soil content to the different phosphorus supply was studied. The crop yields, B content in plants, B-uptake, and content of B (extracted by Mehlich 3 and NH_4 acetate methods) were determined. Spring barley and winter wheat B uptake was about one order of magnitude lower in comparison with oilseed rape. Significant differences in B content in soils, in crop tissues and B-uptake, were found mainly under higher phosphorus doses (40 and 80 kg P/ha). NH_4 acetate method showed better correlations between P and B contents in soils than Mehlich 3 method from the second experimental year. The P-fertilization may affect negatively the B-uptake by plants, particularly if the highly nutrient demanding crop is grown.

Keywords: nutrition; micronutrient; soil testing; boron deficiency; extraction procedure

Boron (B) represents one of the most important micronutrients necessary for plant growth which can be limited in high or low B concentrations (i.e., Camacho-Cristóbal et al. 2011, Davies et al. 2011).

Boron availability in soils is regulated by its equilibrium concentration that in turn is buffered by adsorption and desorption reactions. Boron is associated with various soil components, including adsorbed/exchange forms and those occluded in mineral phases (clays and Fe/Al hydroxides). Ionic strength, pH, organic matter content, intrasoil biological cycle; humification; isomorphic substitution of clay minerals and the formation of colloids and the type and amount of minerals are the major factors affecting B sorption reactions (Majidi et al. 2010, Kot 2015). Dissolved B compounds occur as undissociated boric acid and borates in the soil solution, which is supposed to be major bioavailable forms of B (Hu and Brown 1997, Kot 2015). Other important factors affecting B availability are soil texture and moisture, temperature and carbonates (Zhu et al. 2007, Matula 2009). The formation of borate-diol complexes with a variety of organic molecules was described by Hu and Brown (1997) and Hu et al. (1997). Boron deficiency has been reported in many regions in the Americas, Europe, Africa, and Asia and can cause serious problems in many of the world's

growing areas due to yield losses (Rerkansem et al. 2004). Similarly, the Czech Republic has generally low B-content in agricultural soils (Matula 2009).

For many years it was thought that the passive diffusion had played the main role in B-uptake by plants, however B transporters are already known (Camacho-Cristóbal et al. 2011). Boron has a narrow range between deficiency and toxicity (i.e., Matula 2009). The degree of B toxicity symptom expression can vary in different wheat cultivars (Kalayci et al. 1998); the wheat sterility at B deficiency enhanced by drought was described by Pant et al. (1998). Tolerance to high or low B-contents among barley and wheat cultivars differing in B concentration, e.g. in grain at maturity also described Cartwright et al. (1985). The phenotype of plant response to low B is more complex in barley than wheat and may require different strategies for managing B nutrition in barley including different approaches for selecting B efficient genotypes (Wongmo et al. 2004). The decline in residual contents of B from a single fertilizer addition was closely related to the concentration of B in the soil and oilseed rape leaf. The content of available B in the soil also decreased with the intensive crop rotation (Yang et al. 2000).

The interactions between boron and phosphorus were described already by Kaya et al. (2009) who found that high B-contents reduced phosphorus contents in plant leaves. Also, Mühlbachová et al. (2017) in a pot experiment showed the decrease of B-contents in soils under increasing P rates.

The aim of a three-year field experiment was to evaluate changes in: (i) available B-contents in soils under increasing P doses; (ii) crop yields, B concentrations in used crops (spring barley, winter oilseed rape, and winter wheat) and B-uptake under increasing P fertilization rates; (iii) relationships between B and P contents in soils, obtained crop yields, B contents in plants and B-uptake.

MATERIAL AND METHODS

Field experiment. The three-year field experiment with increasing rates of phosphorus was carried out in the years 2015–2017 at the Humpolec experimental station (Bohemian-Moravian Highlands, Czech Republic, 49°33'N, 15°33'E), altitude 525 m a.s.l. The soil type is Gleic Cambisol with sandy-loam texture. The basic soil contents based on Mehlich 3 method are: 113 mg K/kg, 115 mg Mg/kg, 2255 mg Ca/kg. The phosphorus contents in soils are given in Table 1. The crop rotation was: spring barley (cv. KWS Irina)-winter oilseed rape (cv. Basalti)-winter wheat (cv. Elly). The nitrogen was added uniformly in CAN (27% N) form in all experimental treatments: 30 kg N/ha in the year 2015, 160 kg N/ha in 2016 and 130 kg N/ha in 2017. The phosphorus was applied as triple superphosphate (20% P) in the following rates: control (0 P/ha); P-20 (20 kg P/ha); P-40 (40 kg P/ha) and P-80 (80 kg P/ha) in all years of experiment. Each treatment was four times replicated every experimental year. The experimental plots were 21 m^2 (6 × 3.5 m); regularly ploughed to 0.22 m. Crops were sprayed with insecticides and herbicides according to standard agrotechnics.

Each experimental plot was individually harvested at the phase of maturation of given crop. The yield of the main product (grain, seeds) and of the straw was determined. Simultaneously, the soil samples were taken from the depth of 0–30 cm from 8 places.

Plant and soil analysis. The exchangeable nutrient contents in soils were determined by the NH$_4$-acetate method (Matula 2007). The contents of available nutrients were determined according to the Mehlich 3 method (Mehlich 1984) in a slight modification of Trávník et al. (1999). More detailed information was described by Mühlbachová et al. (2017). Boron contents in plants were deter-

Table 1. The phosphorus (P)-Mehlich 3 and P-NH$_4$-acetate contents (mg/kg soil) in soils in the field experiment

Treatment	P-Mehlich 3			P-NH$_4$-acetate		
	2015	2016	2017	2015	2016	2017
Control	88.3 ± 15.1[a]	89.0 ± 14.5[a]	80.6 ± 11.2[a]	25.0 ± 8.9[b]	14.5 ± 3.1[a]	14.4 ± 3.6[a]
P-20	89.4 ± 11.6[a]	99.1 ± 16.6[a]	90.5 ± 17.7[a]	24.9 ± 3.4[b]	17.4 ± 2.3[b]	16.2 ± 3.9[ab]
P-40	88.3 ± 16.0[a]	95.1 ± 5.8[a]	93.6 ± 4.0[a]	23.4 ± 6.1[ab]	16.2 ± 1.5[ab]	17.0 ± 0.4[ab]
P-80	102.3 ± 20.0[a]	110.4 ± 8.6[b]	102.3 ± 24.0[a]	21.3 ± 4.3[ab]	21.2 ± 4.0[ab]	18.4 ± 4.3[ab]

Different letters indicate significant differences according to the Tukey's test in individual years. Control – 0, P-20 – 20, P-40 – 40, P-80 – 80 kg P/ha

Table 2. The grain/seed and straw yields (t/ha) in the field experiment

Treatment	Yield grain/seed			Yield straw		
	barley	oilseed rape	wheat	barley	oilseed rape	wheat
	2015	2016	2017	2015	2016	2017
Control	8.47 ± 0.27[a]	2.51 ± 0.07[a]	9.05 ± 0.34[a]	5.09 ± 0.29[ab]	4.24 ± 0.11[ab]	3.89 ± 0.16[a]
P-20	8.67 ± 0.17[ab]	2.41 ± 0.11[a]	9.02 ± 0.21[a]	5.27 ± 0.2[b]	4.19 ± 0.12[ab]	3.89 ± 0.16[a]
P-40	8.52 ± 0.20[a]	2.65 ± 0.09[a]	9.01 ± 0.14[a]	5.14 ± 0.12[ab]	4.58 ± 0.12[bc]	3.81 ± 0.16[a]
P-80	8.78 ± 0.22[ab]	2.83 ± 0.1[a]	9.08 ± 0.18[a]	5.42 ± 0.15[b]	4.80 ± 0.26[cd]	3.85 ± 0.13[a]

Different letters indicate significant differences according to the Tukey's test in individual years. Control – 0, P-20 – 20, P-40 – 40, P-80 – 80 kg P/ha

mined separately in grain or seeds and in the straw after digestion in concentrated nitric acid and 30% hydrogen peroxide by use of microwave Milestone 1200 (Connecticut, USA). The nutrient contents in extracts were determined by the ICP-OES Thermo Jarrel Ash (Nebraska, USA). Possible memory effects were avoided by regular rinsing with deionized water after each sample and by instrument recalibration after 10 individual measurements. The overall uptake of nutrients was calculated from the yields and the nutrient contents in grains and straw of each crop.

Statistical analysis. The results from the overall period 2015–2017 were statistically analysed using the Statistica 13.0 software (TIBCO Software, Palo Alto, USA). The one-way (treatments) and two-way (treatments and years) ANOVA and the Tukey's test were used to determine significant differences among the years and treatments. The correlation coefficients (*r*) based on Spearman's equations were calculated.

RESULTS AND DISCUSSION

Crop yields. Despite numerous reports about positive effects of phosphorus on crop growth and yields (i.e. Davies et al. 2011) and the fact that the yields of the spring barley and oilseed rape increased under P fertilization, no significant relationship between treatments for the yields of grain or seeds of grown crops were observed (Table 2). The spring barley yields varied between 8.47 t/ha in control treatment and 8.78 t/ha in P-80 treatment. Similarly, the yield of oilseed rape varied between 2.41 t/ha in P-20 treatment to 2.83 t/ha in P-80 treatment. The winter wheat yields varied only slightly (9.01–9.08 t/ha), and effects of different P treatments were not significant. A significant effect of P treatments was observed mainly for the straw yield of oilseed rape ($P \leq 0.01$) (Table 4) which responded positively to the P-80 treatment.

B-content in plants. The B contents in spring barley grain (1.19–1.67 mg B/kg) and winter wheat (0.69–1.07 mg B/kg) decreased significantly with the increasing phosphorus dose ($P \leq 0.01$). According to the Tukey's test, a significant decrease of B-contents was noted at P-80 dose in spring barley and at P-40 and P-80 doses in winter wheat. B-contents in the straw of cereals tended to decrease under increasing P doses, but no significant effect was observed. A slight tendency to decrease B contents with increasing P rates was noted in oilseed rape seeds, but no statistical difference among treatments was found (Table 4). The observed differences in B concentrations in cereal and oilseed rape straws possibly indicate different mechanisms of B-uptake.

B-uptake. The B-uptake under increasing P doses decreased significantly in grain and straw

Table 3. The boron (B) Mehlich 3 and B NH_4-acetate contents (mg/kg soil) in soils in the field experiment

Treatment	B-Mehlich 3			B-NH_4-acetate		
	2015	2016	2017	2015	2016	2017
Control	1.28 ± 0.06[c]	1.07 ± 0.04[b]	0.94 ± 0.08[ab]	0.31 ± 0.02[bc]	0.36 ± 0.05[d]	0.39 ± 0.06[d]
P-20	1.27 ± 0.07[c]	1.05 ± 0.05[ab]	0.92 ± 0.07[ab]	0.29 ± 0.02[abc]	0.26 ± 0.03[ab]	0.27 ± 0.03[ab]
P-40	1.31 ± 0.05[c]	1.06 ± 0.04[ab]	0.92 ± 0.05[ab]	0.28 ± 0.01[ab]	0.23 ± 0.02[ab]	0.25 ± 0.02[abc]
P-80	1.29 ± 0.09[c]	1.01 ± 0.08[ab]	0.89 ± 0.12[a]	0.27 ± 0.02[ab]	0.18 ± 0.02[a]	0.22 ± 0.02[ab]

Different letters indicate significant differences according to the Tukey's test in three years of experiment. Control – 0, P-20 – 20, P-40 – 40, P-80 – 80 kg P/ha

Table 4. The boron contents (mg/kg dry matter) in grain/seeds and straw of crops grown in the field experiment

Treatment	B grain/seed			B straw		
	barley	oilseed rape	wheat	barley	oilseed rape	wheat
	2015	2016	2017	2015	2016	2017
Control	1.67 ± 0.17[b]	11.91 ± 0.77[c]	1.07 ± 0.10[ab]	4.58 ± 0.79[a]	26.00 ± 4.22[c]	1.98 ± 0.38[a]
P-20	1.58 ± 0.13[b]	11.88 ± 0.42[c]	0.91 ± 0.03[ab]	4.25 ± 1.37[a]	19.95 ± 2.17[b]	1.94 ± 0.10[a]
P-40	1.25 ± 0.16[ab]	11.42 ± 0.42[c]	0.75 ± 0.03[a]	3.50 ± 0.95[a]	21.16 ± 1.51[b]	1.77 ± 0.26[a]
P-80	1.19 ± 0.14[a]	11.44 ± 0.37[c]	0.69 ± 0.06[a]	3.39 ± 0.37[a]	20.05 ± 1.39[b]	1.71 ± 0.09[a]

Different letters indicate significant differences according to the Tukey's test in individual years. Control – 0, P-20 – 20, P-40 – 40, P-80 – 80 kg P/ha

of spring barley and winter wheat (Table 5). These results are more consistent to Kaya et al. (2009) who showed that phosphorus could mitigate the symptoms of B toxicity in tomatoes in excess B. However, Mühlbachová et al. (2017) in a pot experiment showed that B-uptake of barley increased with the increasing phosphorus dose. B-uptake by straw of evaluated crops also decreased with increasing P rates. However, the significant effect ($P \le 0.05$) was noted only in the oilseed rape straw. Oilseed rape is sensitive to the deficiency of B in soils (Shi et al. 2009). In our field experiment, it was observed that the oilseed rape B-uptake was higher in comparison with spring barley and winter wheat B-uptake and it was accompanied with a significant decrease of B-content in soil ($P \le 0.01$).

Relationships between crop yields, B-contents in soils, B-contents in plants and B-uptake. No significant correlations were found between the yields of cereals and B-contents in soils, in plants and B-uptake suggesting that boron was not the driven element for obtaining of yields. The grown cereals grain and straw contained about one order of magnitude less B in comparison with the oilseed rape, for which significant correlations were found between yield of seeds and yield of straw and B-NH_4 acetate contents in soils (seeds:

$r = -0.568$, $P \le 0.05$; straw: $r = -0.642$, $P \le 0.05$) and between B-uptake and seed yield ($r = 0.803$, $P \le 0.001$). Correlations between B-uptake and B-content in cereals grain and straw were found for the spring barley grown in the first experimental year and for the winter wheat followed the oilseed rape showing close relationships between these characteristics (Table 6). Yang et al. (2009) revealed the important role of B for the crop physiology and its involvement in the synthesis of cell wall, structure and membrane stability and Rerkasem et al. (2004) showed that B deficiency negatively affected the development of pollen, florets, ears in winter wheat, triticale and durum wheat.

The boron content in soils. The Mehlich 3 method showed B-contents in soils significantly decreased in all experimental years and treatments ($P \le 0.001$) (Table 3). In the 2017, B-content extracted with this method was from 26% to 30% lower than in 2015. However, the Tukey's test did not show significant differences in B-contents determined by Mehlich 3 method within treatments in individual years.

The NH_4-acetate method showed B-contents in soils decreased significantly during the three-year period with increasing P doses up to 44.6% for P-80 treatment and it was significant for the year ($P \le 0.01$)

Table 5. The boron uptake (g/ha) by grain/seeds and straw of examined crops in the field experiment

Treatment	Grain/seed			Straw		
	barley	oilseed rape	wheat	barley	oilseed rape	wheat
	2015	2016	2017	2015	2016	2017
Control	13.4 ± 1.7[d]	95.3 ± 1.7[ab]	8.6 ± 0.9[bc]	36.64 ± 4.8[a]	208.0 ± 17.1[c]	15.84 ± 1.5[a]
P-20	12.6 ± 1.1[cd]	95.0 ± 1.3[ab]	7.3 ± 0.4[abc]	34.00 ± 6.4[a]	159.6 ± 9.2[b]	15.52 ± 0.5[a]
P-40	10.0 ± 1.4[ab]	91.4 ± 1.9[a]	6.0 ± 0.3[ab]	28.00 ± 4.8[a]	169.3 ± 6.2[b]	14.16 ± 1.1[a]
P-80	9.5 ± 1.1[a]	91.5 ± 1.7[a]	5.5 ± 0.5[a]	27.12 ± 2.3[a]	160.4 ± 9.2[b]	13.68 ± 0.5[a]

Different letters indicate significant differences according to the Tukey's test in individual years. Control – 0, P-20 – 20, P-40 – 40, P-80 – 80 kg P/ha

Table 6. Correlation coefficients between phosphorus (P) and B-NH$_4$ acetate extractable content in soils, boron (B) content in crops and B-uptake

Treatment	B-NH$_4$ acetate	P-NH$_4$ acetate	B-grain/ seed	B-straw	B-uptake grain/seed	B-uptake straw	B-uptake total
Spring barley							
B-Mehlich 3	ns	ns	ns	ns	ns	ns	ns
B-NH$_4$ acetate	–	ns	ns	ns	ns	ns	ns
B-grain/seed	0.561*	ns	–	ns	0.989***	ns	0.636**
B-straw	0.546*	ns	–	–	ns	0.913***	0.948***
B-uptake grain	ns	0.577*	–		–	ns	0.663**
B-uptake straw	ns	ns	–	–	–	–	0.894***
B-uptake total	ns	0.646*	–	–	–		–
Oilseed rape							
B-Mehlich 3	0.575*	ns	ns	ns	ns	ns	ns
B-NH$_4$ acetate	–	−0.722**	0.517*	0.523*	ns	ns	ns
B-grain/seed	ns	ns	–	ns	ns	ns	ns
B-straw		ns	–	–	ns	0.908***	0.857***
B-uptake grain	ns	ns	–	–	–	ns	ns
B-uptake straw	ns	ns	–	–	–	–	0.99***
B-uptake total	ns	ns	–	–	–	–	–
Winter wheat							
B-Mehlich 3	0.556*	ns	ns	ns	ns	ns	ns
B-NH$_4$ acetate	–	−0.533*	0.889***	0.601*	0.897***	0.575*	0.845***
B-grain/seed	0.888***	ns		0.605*	0.991***	0.579*	0.908***
B-straw	0.600*	ns	–	–	0.634**	0.972***	0.852***
B-uptake grain	0.896***	ns	–	–	–	0.629**	0.937***
B-uptake straw	0.575*	ns	–	–	–	–	0.861***
B-uptake total	0.844***	ns	–	–	–	–	–

ns – not significant; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

and also for the phosphorus dose applied in soil ($P \leq 0.001$). During the first experimental year, the decrease of NH$_4$-acetate B-contents in soils with corresponding P doses was not significant ($P = 0.063$), but in the second and third year it decreased significantly at $P \leq 0.001$ (Table 3).

The Mehlich 3 B-contents in soils showed a clear decrease within the studied years and treatments. No clear effect was observed during the year 2015. The second experimental year when oilseed rape was grown was probably determining for the changes of B contents in soils. Oilseed rape took a bigger amount of B in comparison with the spring barley (Table 4) which could affect the Mehlich 3 B-contents in soils.

The NH$_4$-acetate extracts typically the exchangeable elements from soils, whereas Mehlich 3 can extract also potentially available elements from soil sorption complex. The release of B adsorbed on clay minerals, Al or Fe hydroxides (Hu and Brown 1997, Matula 2009) is not excluded under the higher nutrient demand of oilseed rape, and this effect could persist to the last experimental year when also Mehlich 3 B-contents were lowest. The NH$_4$ acetate soil B-contents decreased mainly under increasing P rates irrespective to the year of experiment suggesting that readily B fractions were associated more with the particular P treatment.

The correlations between P and B contents in soils. No significant correlation was found between P and B content extracted by Mehlich 3 method in none of the studied years in the field experiment. In the case of Mehlich 3 B-contents, an important role could play the presence of less available B fractions complexed on Fe or Al hydroxides or clay minerals (Matula 2009, Černý et al. 2016). The boron fractions determined by NH$_4$ acetate showed from the second experimental year significantly negative

2015

$y = 0.000x + 0.269$

$R^2 = 0.034$

$r = 0.184$, ns

2016

$y = -0.014x + 0.491$

$R^2 = 0.522, r = -0.722, P \leq 0.01$

2017

$y = -0.009x + 0.426$

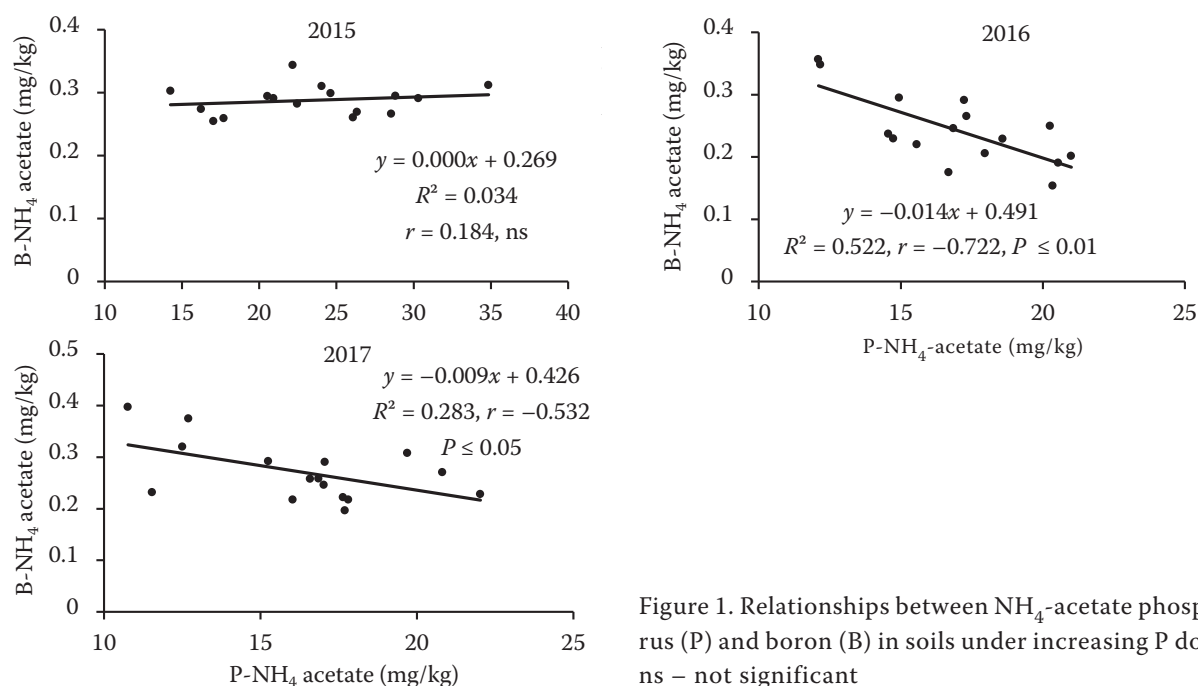$R^2 = 0.283, r = -0.532$

$P \leq 0.05$

Figure 1. Relationships between NH_4-acetate phosphorus (P) and boron (B) in soils under increasing P doses. ns – not significant

correlations between P and B-contents (Figure 1). Such result may be caused by the possibility that the more readily available B fractions were more predictive to changes of phosphorus contents in soils. Similarly the correlations between B-Mehlich 3 and B-NH_4 acetate contents that were observed from the second year of the experiment (Figure 2) suggesting that the B-uptake by grown crops and particularly the oilseed rape were responsible for the

decrease of B-contents in soils under increasing P rates. The following factors could play an important role in the availability of B in soils with different P fertilization: (1) the B-uptake by oilseed rape is demanding more B in comparison with cereals; (2) the possible B leaching to lower parts of soil profile and consequently to ground waters; (3) the interaction was found between P and B in soils as was suggested by Mühlbachová et al. (2017) and Kaya et al. (2009).

2015

$y = -0.0717x + 0.3801$

$R^2 = 0.0393$

$r = 0.1$, ns

2016

$y = -0.014x + 0.491$

$R^2 = 0.522, r = -0.722; P \leq 0.01$

2017

$y = -0.009x + 0.426$

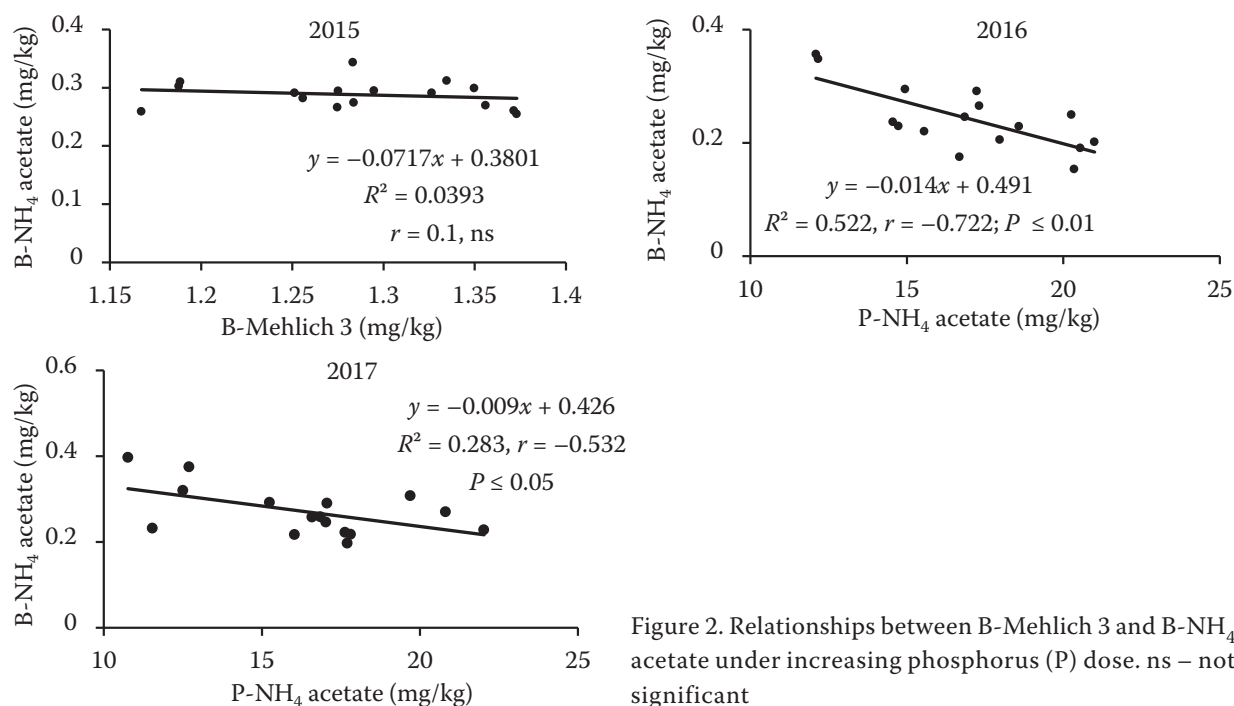$R^2 = 0.283, r = -0.532$

$P \leq 0.05$

Figure 2. Relationships between B-Mehlich 3 and B-NH_4 acetate under increasing phosphorus (P) dose. ns – not significant

The crop yields increased only partly under higher P doses and mostly non-significantly. The boron concentrations under increased P doses tended to decrease in crop tissues which consequently led to the decrease of B-uptake in a given crop. The oilseed rape demanding much more B grown in the second year of an experiment than cereals could be one of the important factors decreasing B contents in soils. The determination of boron by two methods showed that the Mehlich 3 method tended to decrease B contents under higher B-uptake by crops. The second method – NH_4 acetate – was able to show the differences in B content under increased P doses. Increasing doses of phosphorus decreased B content in soils. This effect was the most pronounced in the second year of the experiment when oilseed rape, demanding more nutrients, was grown. This effect can persist in the following vegetation season as shown in the results obtained in the third year of the experiment. The attention should be paid to boron fertilization of crops with higher nutrient supply needs.

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