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Efficacy of ^{15}N-nitrogen in fertilization of pea mixtures with wheat, barley, and oats

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ABSTRACT

The effect of mineral nitrogen (N) nutrition on seed production and acquisition of ^{15}N from fertilizers by components of cereal-pea mixtures was investigated. Yields of wheat-pea and oats-pea mixtures raised together with the increase of cereals percentage in the pot, and higher seed yields of mixture with barley was affected by a larger share of pea plants. The percentage of nitrogen derived from fertilizers was significantly higher in cereals as compared with pea. Pea accumulated the greatest quantity of nitrogen from fertilizers in straw and roots, and cereals translocated ^{15}N mainly to ears – grain and glumes with rachis. The percentage of ^{15}N in seeds of pea amounted to 15% on the average, and in grain of cereals – 54% to 60% of the total N taken up.

Keywords: cereal-pea mixtures; seeding rate; isotopic method; N distribution within plant

Intercropping of cereals and grain legumes is a practice intended to stabilize yields, make better use of plant growth resources and enhance biodiversity. Due to differences in architecture of root system, physiology and growth cycle, these species in mixtures use greater quantity of a resource and utilize it more efficiently than if they are grown alone (Hauggar-Nielsen et al. 2001). It is often assumed that a portion of the N_2 fixed by an intercropped legume is made available to the associated non-legume (Fujita et al. 1990).

Fertilization of mixtures in order to provide the cereal component with nitrogen (N) is the critical in cereal-legume growing. There is a common belief that higher doses of nitrogen fertilizers are not economically justifiable as they depress atmospheric N fixation by legumes, and they prolong the vegetation period (Nesheim et al. 1990, Filek et al. 1997). The growth and development of cereal plants are dependent on availability of nitrogen throughout the whole vegetation period. Field pea needs a little amount of nitrogen until the symbiosis with *Rhizobium* is induced. For the flowering and pods formation, pea plants utilize N_2 biologically fixed, and this is sufficient to meet their N needs (Vessey 1992). The literature shows the increasing in N rates applied to mixtures led to the cereal yield rise, and did not affect the legume yield (Borowiecki and Księżak 2000). However, the fate of nitrogen applied in the form of mineral fertilizers as well as its utilization by legume and cereal components under different seeding ratio is not well recognized. Moreover, accumulation of nitrogen from mineral fertilizers by legume and its distribution to vegetative and generative parts of plant seems to be crucial for nitrogen fertilization of the mixtures. The real amount of N from fertilizers accumulated by the components of mixtures is possible to access only by using isotopic method.

The aim of this paper was to evaluate the effect of mineral N fertilization on seed yield, protein yield and utilization of ^{15}N from fertilizers by components of field pea mixtures with wheat, barley and oats, at a different seeding ratio.

MATERIAL AND METHODS

Pot experiments were carried on in 2010 and 2011 at the greenhouse in the Experimental Station in Puławy of the Institute of Soil Science and Plant

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Cultivation, Poland. The Mitscherlich's pots were filled with 6.0 kg sandy loam soil mixed with 1.0 kg sand, supplemented with optimal rates of macro- and micronutrients. The total content of nitrogen and organic carbon in the soil amounted to 0.6 g/kg soil and 6.2 g/kg soil, respectively, and pH value measured in KCl was equal to 6.6. The content of mineral nitrogen was below 10 mg/kg soil. The experiments were set up in a completely randomized block design with three replications. The first factor was the species of cereals in the mixture with field pea-wheat (cv. Ismena), barley (cv. Rataj) or oats (cv. Jawor), and the second factor – the proportion of cereal component in the mixture – 0, 33, 57, 75, 88 and 100%. The rates of nitrogen fertilizers in the form of $^{15}NH_4{}^{15}NO_3$ (10 at% excess) were fitted to the number of cereal plants in the pot. The soil moisture was adjusted to 50–60% of its field capacity. Five pea plants and ten plants of wheat, barley, and oats in the pot were taken as the optimal plant density in the pure stand. The percentage of cereal component, and adequate nitrogen rates were presented in Table 1.

Total nitrogen rates were subdivided into 0.3 g N/pot of which the first was applied before sowing, the second after plant thinning, and the subsequent rates in 7 days intervals. At maturity, aboveground biomass was harvested, separated into grain, rachis with glumes or pods, and straw. Roots were separated from soil. Plant samples were dried at 60°C and the dry matter of all plant organs was evaluated.

Total nitrogen concentration in plant samples, as well as ^{15}N analysis were performed by the means of elemental analyzer – isotope ratio mass spectrometer (PDZ Europa ANCA-GSL elemental analyser interfaced with a Sercon 20-20 IRMS with SysCon electronics (SerCon, Cheshire, UK)) in Department of Applied Analytical and Physical Chemistry, Isotope Bioscience Laboratory, Gent University in Belgium. Protein content was calculated from the percentage of total nitrogen using a conversion factor – (%N × 5.75 for cereals and 6.25 for pea).

Statistical processing of the results was performed using the Statgraphics 5 Plus package (Statgraphics Plus, Rockville, USA). The Tukey's test has been applied to evaluate the significance of differences between the treatments.

Table 1. Percentage of cereals, number of plants in the mixture, and nitrogen rate per pot (g)

Percentage of cereals in the mixture	Number of plants in the pot		Nitrogen rate
	cereal	field pea	
100	10	0	1.5
88	8	1	1.2
75	6	2	0.9
57	4	3	0.6
33	2	4	0.3
0	0	5	0

RESULTS AND DISCUSSION

Yields of cereal-pea mixtures. The mean yields of pea with wheat reached 60.0 g, with barley 43.2 g, and with oats 53.9 g per pot. Similarly to studies of Lithourgidis et al. (2011), on pea intercropped with cereals, yields of wheat-pea and oats-pea mixtures raised together with the percentage of cereals in the pot. On the contrary, higher seed yields of mixtures with barley were affected by a greater share of pea plants. Thus, wheat and oats in a pure stand produced more seeds as compared with their mixtures with pea, and pea grown alone (Figure 1).

As the literature shows (Izzauralde et al. 1990, Carr et al. 1998) various species of cereals differ in the degree of dominance in mixed crops with

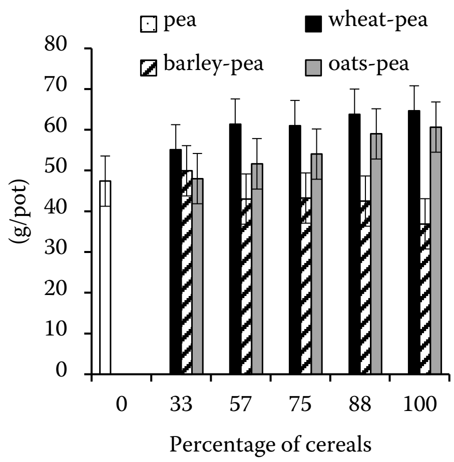

Figure 1. Seed yields of cereal-pea mixtures and grown in a pure stand. 0% – pure stand of pea; 100% – pure stand of wheat, barley or oats

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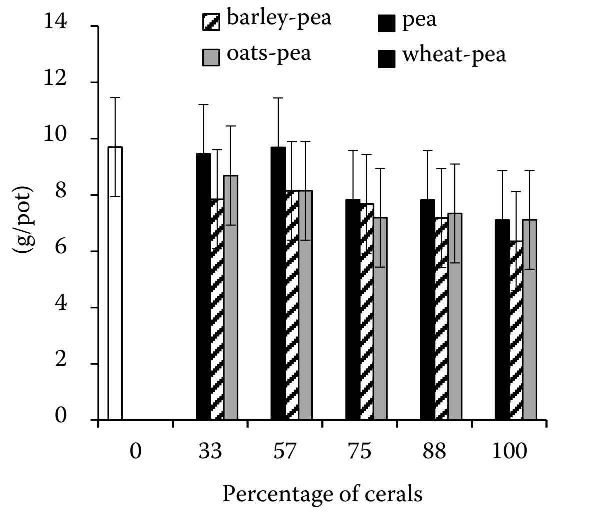

Figure 2. Protein yields of cereal-pea mixtures and grown in a pure stand. 0% – pure stand of pea; 100% – pure stand of wheat, barley or oats

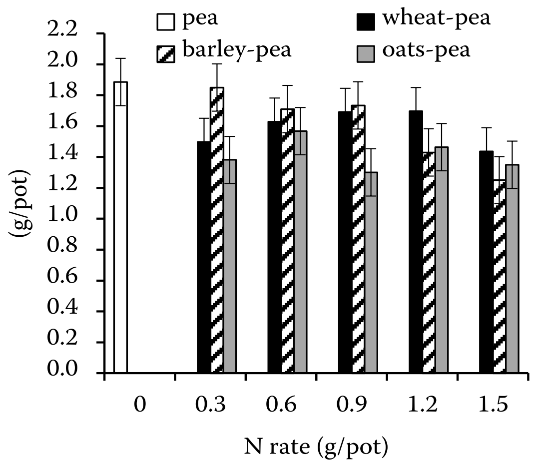

Figure 3. Nitrogen uptake by cereal-pea mixtures and grown in a pure stand. 0% – pure stand of pea; 100% – pure stand of wheat, barley or oats

pea. The most competitive is oats and wheat, and the less is barley (Neugschwandtner and Kaul 2015). In the experiment, in stand with barley, the proportion of pea seeds in the mixture was about 30% greater than its seeding ratio. The proportion of wheat and oats seeds in the final yield was comparable to the percentage of cereal plants in the pot.

Protein yield. Nitrogen fertilization increased grain N concentration of cereals but did not alter pea grain N content. The highest protein yield was obtained in pea monoculture and in wheat-pea mixture at 33% and 57% of cereals (Figure 2). Protein yield of mixtures was always higher than cereals' in a pure stand, and there was an increase in protein yield as the pea proportion raised, which is reported in other studies (Carr et al. 2004, Lithourgidis et al. 2011).

Nitrogen uptake by cereal-pea mixtures. Total nitrogen uptake was calculated as the sum of N uptake by biomass of mixtures or by pea and cereals in a monoculture. Pea in a pure stand took up 1.88 g N/pot, mixtures with cereals about 17% less compared to pea, and cereals in a pure stand about 28% less to the legume. Nitrogen uptake was determined by the number of pea plant in a pot. In the treatment 0.3 g N/pot, which consists of four pea plants and two cereal plants, mixtures accumulated 1.60 g of nitrogen on the average which is over five times more than applied in fertilizers (Figure 3).

Nitrogen derived from fertilizers (%Ndff). Of the total pool of nitrogen, the percentage of N derived from fertilizers applied to mixtures was significantly higher in cereals as compared with pea (Table 2). For both, cereals and pea, the

Table 2. Nitrogen derived from fertilizers (%Ndff) in plant organs of pea and cereals grown in the mixtures

Plant organ	Cereal-pea mixture					
	wheat	pea	barley	pea	oats	pea
Grain/seed	56^{a}	15^{a}	60^{a}	16^{a}	60^{a}	17^{a}
Glumes and rachis/pods	52^{a}	19^{a}	58^{a}	22^{a}	59^{a}	22^{ab}
Stalk and leaves	50^{a}	29^{b}	56^{ab}	27^{a}	55^{ab}	33^{bc}
Roots	44^{b}	26^{b}	48^{b}	26^{a}	48^{b}	31^{c}

Treatments with the same letter are not significantly different ($P \leq 0.05$)

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nitrogen nutrition regime had a strong effect of this index. Regardless of the N dose, pea accumulated the greatest quantity of nitrogen from fertilizers in straw (stalk with leaves), pods and in roots. According to results obtained by Schiltz et al. (2005) on dynamics of nitrogen in pea organs, remobilization of endogenous nitrogen from vegetative parts contributed to 71% of the total N in mature seeds, and it is a unique pool managed at the whole plant level. Thus, exogenous nitrogen, not distributed to seeds is accumulated in the vegetative plant parts. On the contrary, cereals translocated nitrogen from fertilizers mainly to ears – grain and glumes with rachis (Rutkowska 2009, Wysokiński et al. 2015). The percentage of ^{15}N in seeds of pea amounted to 15% on the average, and in grain of cereals – 54% to 60%.

Uptake and utilization of nitrogen from fertilizers. The quantity of ^{15}N from fertilizers taken up by plants was calculated using the isotope dilution method (IAEA 2008). Irrespective of species composition, the amount of ^{15}N uptake by mixtures raised together with nitrogen rate, and simultaneously with the number of cereal plants (Table 3). Therefore, cereals as sole crops showed the highest value of ^{15}N uptake. Barley grown with pea accumulated the greatest quantity of total nitrogen, but had the lowest proportion of ^{15}N (34% on the average). Oats as an intercrop showed the greatest share of nitrogen from fertilizers in the total pool of nitrogen accumulated in the above groundmass (39% on the average).

The cereal component did not determine N uptake from fertilizers by field pea, which used about 18% of the total pool of N accumulated at maturity in aboveground biomass. Accumulation of ^{15}N by cereals was strongly affected by nitrogen fertilization. Nitrogen from fertilizers taken up by wheat as the intercrop accounted for 49% of the total N pool accumulated in aboveground biomass, by barley and oats – 55% on the average. As the monocrops, the proportion of ^{15}N in wheat biomass reached 61%, in barley and oats – 71% on the average.

Based on Tables 1 and 3, the quantity of total N and ^{15}N taken up by components of the mixtures was recalculated on an individual pea and cereal plant. While the amount of total N accumulated by a single pea plant in the mixtures was not affected by a nitrogen regime, the acquisition of ^{15}N from fertilizers strongly raised with N doses, from 0.028 to 0.10 g ^{15}N. On the contrary, a cereal plants took almost the same quantity of ^{15}N from fertilizers (0.07–0.09 g ^{15}N average). It is documented that field pea which can choose between mineral nitrogen and nitrogen fixed from air used mineral N, because carbon costs of nitrogen fixation are higher than those associated with mineral N assimilation (Buttery and Gibson 1990). However, taking into account the total quantity of nitrogen accumulated by pea plant grown as a component of the mixtures, there has been no disadvantage resulting from mineral fertilization. Irrespective of nitrogen availability, pea plant accumulated almost the same quantity of total N (0.30–0.35 g). To compare, an individual pea plant grown as the monocrop, in absence of mineral fertilizers it accumulated 0.37 g N. The impact of fertilization of cereal-legume mixtures with nitrogen on biological nitrogen fixation is inconsistent and depends

Table 3. Total nitrogen uptake ($N_{tot,}$ g/pot) and ^{15}N uptake ($^{15}N_{up,}$ g/pot) by wheat, barley and oats in the mixtures

N dose (g/pot)	Cereal-pea mixture											
	wheat		pea		barley		pea		oats		pea	
	N_{tot}	$^{15}N_{up}$	N_{tot}	$^{15}N_{up}$	N_{tot}	$^{15}N_{up}$	N_{tot}	$^{15}N_{up}$	N_{tot}	$^{15}N_{up}$	N_{tot}	$^{15}N_{up}$
0.3	0.39[a]	0.14[a]	1.11[a]	0.15[a]	0.36[a]	0.16[a]	1.48[a]	0.13[ab]	0.29[a]	0.12[a]	1.10[a]	0.15[b]
0.6	0.71[ab]	0.34[b]	0.92[ab]	0.14[a]	0.71[b]	0.34[a]	1.00[ab]	0.12[a]	0.68[b]	0.34[ab]	0.89[ab]	0.18[ab]
0.9	1.10[bc]	0.57[c]	0.64[bc]	0.13[a]	0.93[bc]	0.58[b]	0.81[ab]	0.16[b]	0.80[b]	0.52[b]	0.47[bc]	0.12[ab]
1.2	1.31[c]	0.78[d]	0.38[c]	0.10[ab]	1.10[cd]	0.71[bc]	0.40[b]	0.16[ab]	1.21[c]	0.82[c]	0.26[c]	0.08[a]
1.5	1.44[c]	0.89[d]	–	–	1.26[d]	0.89[d]	–	–	1.35[c]	0.95[c]	–	–
Mean	0.73	0.55	0.76	0.14	0.87	0.54	0.92	0.14	0.87	0.55	0.68	0.13

Treatments with the same letter are not significantly different ($P \leq 0.05$)

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Table 4. Coefficient of ^{15}N utilization ($^{15}N_{ut}$, %) by components of the mixtures

N dose (g/pot)	Cereal-pea mixture					
	wheat-pea		barley-pea		oats-pea	
	$^{15}N_{ut\ wheat}$	$^{15}N_{ut\ pea}$	$^{15}N_{ut\ barley}$	$^{15}N_{ut\ pea}$	$^{15}N_{ut\ oats}$	$^{15}N_{ut\ pea}$
0.3	46^a	50^c	53^a	43^c	40^a	50^c
0.6	57^a	23^b	56^a	20^{bc}	56^a	30^c
0.9	63^a	14^b	64^a	17^b	57^a	13^b
1.2	65^a	8^a	59^a	13^a	68^a	6^a
1.5	59^a	–	74^a	–	79^a	–

Treatments with the same letter are not significantly different ($P \leq 0.05$)

on numerous factors. Some authors (Patra et al. 1986, Voisin et al. 2002) suggest that optimal nitrogen nutrition of pea is possible by replacement of symbiotic nitrogen fixation by root mineral absorption. The others state, since cereals seems to be more competitive for soil inorganic nitrogen than legumes due to faster and deeper root growth and higher demand in nitrogen, consequently the legumes usually increase their reliance on symbiotic N_2 fixation (Li et al. 2006).

Coefficient of ^{15}N utilization by the mixtures of the rate 0.3 g N/pot was very high and reached 94% on the average (Table 4). Under the lowest nitrogen regime, the coefficient of ^{15}N utilization by pea was similar or higher compared to cereals and decreased together with N rates, which confirms the needs of pea to mineral N nitrogen, in particular at the early vegetation period. On the contrary, the coefficient calculated for cereals raised through N supply and resulted from a higher percentage of cereal plants for which the nitrogen from fertilizer was the main source of this element. In general, utilization of nitrogen from fertilizers in mixtures was always higher compared with its use by cereals as a monocrop and reached 73–96%.

In conclusion, the most optimal percentage of cereal in mixtures with pea was 33% and 57%. The further increase of cereal proportion lead to decline the protein yield and required higher doses of mineral fertilization. Field pea grown together with cereals utilized 23% of nitrogen from fertilizers on average, and accumulated it mainly in straw and roots. On the contrary, cereals recovered about 58%, of which the greatest quantity was stored in grain.

REFERENCES

Borowiecki J., Księżak J. (2000): Legumes in mixtures with cereals in the forage production. Advances of Agricultural Sciences Problem Issues, 2: 89–100. (In Polish)

Buttery B.R., Gibson A.H. (1990): The effect of nitrate on the time course of nitrogen fixation and growth in *Pisum sativum* and *Vicia faba*. Plant and Soil, 127: 143–146.

Carr P.M., Martin G.B., Caton J.S., Poland W.W. (1998): Forage and nitrogen yield of barley-pea and oat-pea intercrops. Agronomy Journal, 90: 79–84.

Carr P.M., Horsley R.D., Poland W.W. (2004): Barley, oat, and cereal-pea mixtures as dryland forages in the Northern Great Plains. Agronomy Journal, 96: 677–684.

Fujita K., Ogata S., Matsumoto K., Masuda T., Ofosu-Budu K.G., Kuwata K. (1990): Nitrogen transfer and dry matter production in soybean and sorghum mixed cropping system at different population densities. Soil Science and Plant Nutrition, 36: 233–241.

Hauggaard-Nielsen H., Ambus P., Jensen E.S. (2001): Interspecific competition, N use and interference with weeds in pea-barley intercropping. Field Crops Research, 70: 101–109.

Filek W., Kościelniak J., Grzesiak S. (1997): The effect of nitrogen fertilization and population density of the field bean (*Vicia faba* L. *minor*) of indeterminate and determinate growth habit on the symbiosis with root nodule bacteria and on the seed yield. Journal of Agronomy and Crop Science, 179: 171–177.

International Atomic Energy Agency (IAEA) (2008): Guidelines of Nitrogen Management in Agricultural Systems. Vienna, IAEA, 62–63.

Izaurralde R.C., Juma N.G., McGill W.B. (1990): Plant and nitrogen yield of barley-field pea intercrop in cryoboreal – Subhumid central Alberta. Agronomy Journal, 82: 295–301.

Li L., Sun J.H., Zhang F.S., Guo T., Bao X., Smith F.A., Smith S.E. (2006): Root distribution and interactions between intercropped species. Oecologia, 147: 280–290.

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Lithourgidis A.S., Vlachostergios D.N., Dordas C.A., Damalas C.A. (2011): Dry matter yield, nitrogen content, and competition in pea-cereal intercropping systems. European Journal of Agronomy, 34: 287–294.

Nesheim L., Boller B.C., Lehmann J., Walther U. (1990): The effect of nitrogen in cattle slurry and mineral fertilizers on nitrogen fixation by white clover. Grass and Forage Science, 45: 91–97.

Neugschwandtner R.W., Kaul H.-P. (2015): Nitrogen uptake, use and utilization efficiency by oat-pea intercrops. Field Crops Research, 179: 113–119.

Patra D.D., Sachdev M.S., Subbiah B.V. (1986): ^{15}N studies on the transfer of legume-fixed nitrogen to associated cereals in intercropping systems. Biology and Fertility of Soils, 2: 165–171.

Rutkowska A. (2009): ^{15}Nitrogen study on accumulation and allocation of nitrogen applied at anthesis on top of early nitrogen applications in winter wheat. Journal of Plant Nutrition, 32: 1306–1320.

Schlitz S., Munier-Jolain N., Jeudy Ch., Burstin J., Salon Ch. (2005): Dynamics of exogenous nitrogen partitioning and nitrogen remobilization from vegetative organs in pea revealed by ^{15}N *in vivo* labelling throughout seed filling. Plant Physiology, 137: 1463–1473.

Wysokiński A., Kalembasa S., Łozak I. (2015): Dynamics of nitrogen accumulation from various sources by lucerne (*Medicago sativia* L.). Acta Scientarum Polonorum. Agricultura, 14: 97–105.

Vessey J.K. (1992): Cultivar differences in assimilate partitioning and capacity to maintain N_2 fixation rate in pea during pod-filling. Plant and Soil, 139: 185–194.

Voisin A.-S., Salon Ch., Munier-Jolain N.G., Ney B. (2002): Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum sativum* L.). Plant and Soil, 242: 251–262.

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