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Influence of foliar fertilization with amino acid preparations on morphological traits and seed yield of timothy

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ABSTRACT

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The aim of the study was to determine the influence of amino acid fertilizer on yielding ability, morphological characteristics and chlorophyll index of timothy cv. Owacja grown for seed. The experiment was conducted in 2015–2017 at the Experimental Station in Prusy near Krakow, which belongs to the Institute of Plant Production of the University of Agriculture in Krakow. The field trial was set up as a randomized block design with four replications. The soil in the experimental field was a degraded chernozem formed from loess. The plots were sprayed with three doses of Microfert amino acid fertilizer: 1.8, 3.0 and 4.5 L/ha. The highest application rate of foliar fertilization with amino acids (4.5 L/ha) caused a significant ($P \leq 0.05$) increase in seed yield and germination capacity compared with the control plot. Successive years of use also had a significant effect on timothy seed yield, which was the highest during the first year of the study. Morphological characteristics were found to improve (taller and longer inflorescences and leaf blades). Satisfactory results (seed yield higher by about 11% to the control plot) were also obtained on the plots where the fertilizer was applied at the rate of 3.0 L/ha.

Keywords: *Phleum pratense* L.; biostimulant; productivity; leaf greenness index; forage grasses

Amino acids are organic chemical compounds and are the building blocks of proteins, which perform structural, metabolic and transport functions in plants (Liu et al. 2008). Amino acids are the precursors of phytohormones and other growth substances. Amino acids improve the efficiency of the plant's metabolism to induce yield increases and enhance crop quality, increasing plant tolerance to and recovery from abiotic stresses, facilitating nutrient assimilation, translocation and use, enhancing quality attributes of product (Calvo et al. 2014), promoting the processes of plant respiration, photosynthesis, protein synthesis, strengthening plant growth and yield formation (Davies 2010). The nitrogen status of plants, and particularly the amino acid pools, is closely linked to photosynthetic activity (Lejay et al. 2003). A special role is played by tryptophan, which is a precursor of auxins – hormones responsible for the rate of stem and root elongation, development of leaf buds, and activity of enzymes. These hormones also regulate protein synthesis. At later developmental stages (maturation), a dominant role is played by methionine, which is a precursor for the biosynthesis of another phytohormone, ethylene. In turn, glycine and glutamic acid are the main substrates for tissue formation and chlorophyll synthesis (Fischer et al. 1998). Their presence contributes to

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chlorophyll production, thus increasing the amount of sugars formed during photosynthesis (Takeuchi et al. 2008). Application of proline with a foliar fertilizer directly before flowering increases the chance of obtaining high yields, because this amino acid influences pollen fertility. Also, lysine, methionine and glutamic acid contribute to pollination, as they stimulate the pollen grain to germinate and activate pollen tube growth (Cao et al. 2010). Because of their full compatibility with the metabolism of fertilized plants, amino acids ensure rapid and easy transport of nutrients into most deficient areas of plants. By increasing the leaf area, chlorophyll content and photosynthesis rate, it is thus possible to obtain higher crop yields of better quality. Studies of the effect of amino acids on increasing crop performance are conducted mainly with vegetables, flowers and ornamental plants, and the results of these experiments confirm the beneficial effect of amino acid fertilization. A positive role of some amino acids in stimulating growth and some chemical components was reported by Talaat et al. (2005) for periwinkle (*Catharanthus roseus* (L.) G. Don), by Abou-Dahab et al. (2006) for red leaf philodendron (*Philodendron erubescens* K.Koch & Augustin), by Nahed et al. (2010) for *Thuja orientalis* L., by Mahgoub et al. (2011) for *Dahlia pinnata* L., and by El-Naggar et al. (2013) for lily (*Lilium* L.). However, no reports are available on the use of amino acids in forage grasses for seed production. Therefore, the aim of this experiment was to determine the effect of amino acid preparation on seed yielding ability, morphological characteristics and selected vegetation indices of timothy grown for seed.

MATERIAL AND METHODS

The experiment was conducted in 2015–2017 at the Experimental Station in Prusy near Krakow, which belongs to the Institute of Plant Production of the University of Agriculture in Krakow. Soil chemical properties were as follows: pH_{KCl} – 6.9, available P – 48.0; K – 132.2 and Mg – 43.6 mg/kg. Total annual precipitation during the study period (2015–2017) ranged from 529.8 mm to 810.4 mm. Average total precipitation during the vegetative period (April–September) was in the range 338.6–573.0 mm. Average annual temperature varied from 9.3°C to 10.2°C during the study years, and from 15.6°C to 16.3°C during the period from April to September.

The field trial was set up as a randomized block design with four replications (plot size of 10 m^2). The plots were sprayed with three doses of Microfert amino acid fertilizer: 1.8, 3.0 and 4.5 L/ha. Four plots were used – a control plot (no fertilizer, only with water) and three experimental plots sprayed with three doses of Microfert® fertilizer: 1.8, 3.0 and 4.5 L/ha. Each dose was divided into three equal parts of: 0.6 L/ha for the dose of 1.8 L/ha, 1.0 L/ha for the dose of 3.0 L/ha, and 1.5 L/ha for the dose of 4.5 L/ha. The first part of the dose was applied after spring growth began, the second at the early stage of stem elongation and the third at the early-heading stage. Spray solutions were prepared by dissolving appropriate amounts of amino acid fertilizer in such a volume of water that the volume of the working liquid corresponded to 300 L/ha. Microfert® amino acid fertilizer is a biostimulant composed of amino acids, peptides and chelated trace elements. The preparation contains 440 g/kg amino acids (including 160 g/kg free amino acids), i.e. alanine 53 g, arginine 2 g, phenylalanine 10 g, glycine 102 g, histidine 3 g, isoleucine 4 g, aspartic acid 20 g, glutamic acid 60 g, leucine 18 g, hydroxylysine 6 g, hydroxyproline 28 g, lysine 22 g, methionine 2 g, ornitine 26 g, proline 50 g, serine 1 g, threonine 2 g, tyrosine 7 g, valine 21 g. Other components of the preparation are: boron 0.63; cobalt 0.013; iron 2.52; manganese 1.26; molybdenum 0.063; zinc 0.13 g/kg. The Microfert® fertilizer used in the experiment is manufactured by Intermag Ltd. in Olkusz. The experiment was conducted with timothy (*Phleum pratense* L.) cv. Owacja. Soil mineral fertilization was applied before sowing using the doses of 50 kg N/ha as ammonium nitrate, 26.2 kg P/ha as triple superphosphate, and 66.4 kg K/ha as 57% potassium salt. In addition, in the autumn the same amounts and forms of phosphorus and potassium were applied as top-dressing before sowing. Similarly, in the years of full use, phosphorus and potassium were applied in the autumn in the same doses and forms. Nitrogen fertilization of timothy was divided into three applications: after harvesting timothy seeds (40 kg N/ha), after spring growth began (20 kg N/ha), and in late April/early May (40 kg N/ha). Timothy seeding rate was 4 kg/ha. The experimental cultivar was sown without a cover crop. The effect of foliar fertilization on chlorophyll content was investigated in each year of the experiment. Leaf greenness index (SPAD) was measured

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with the Minolta 502 SPAD DL (Plainfield, USA) chlorophyll meter on upper leaves. The measurements were performed in each plot on thirty fully developed leaves. Morphological measurements were made on generative shoots at the flowering stage in June. Height of plants, length and width of inflorescences and flag leaf blade were measured. Timothy seeds were harvested between 21 July and the first days of August using a plot combine harvester (NM-ELITE, Wintersteiger, Ried im Innkreis, Austria). Germination capacity was determined separately for each plot using the samples collected after threshing, drying and cleaning. Germination capacity was calculated after 10 days on the Jacobsen apparatus according to standard PN-/R-65950 (ISTA 2010). The results were subjected to an analysis of variance, and significant differences were analysed with the Duncan's test ($\alpha = 0.05$) using Statistica 10 PL (Cracow, Poland).

RESULTS AND DISCUSSION

The present experiment showed that foliar application of the amino acid preparation at different doses (1.8, 3.0 and 4.5 L/ha) had a significant effect on the parameters under study. The morphological characteristics of timothy plants are presented in Table 1. The results from this 3-year study point to a beneficial effect of applying amino acids at the dose of 4.5 L/ha on morphological parameters of timothy. The plants in this plot were taller and produced longer inflorescences and leaf blades compared to the other plots. Likewise, plants in the plots treated with the medium dose also formed wide flag leaves as well as long inflorescences. In plots with no foliar fertilization, the plants produced shorter shoots. Higher growth of plants in response to amino acid application was also reported by Naguib et al. (2003), Youssef et al. (2004) and Nahed et al. (2007). Naguib et al. (2003) demonstrated that application of two amino acids (phenylalanine and methionine) in *Catharanthus roseus* (L.) G. Don caused a considerable increase in plant height, and the higher application rate (100 ppm) was more effective than the lower rate (50 ppm) for both amino acids. Youssef et al. (2004) showed that tyrosine added at 50 ppm to *Ocimum basilicum* L. led to maximum heights of the plant as well as its diameter, number of branches, and fresh and dry matter yield. Also, the application of ornitine, proline and phenylalanine in fertilization of chamomile (*Chamomilla recutita* L.) led to significant increases in the plant height (Nahed et al. 2007). The amino acid fertilization used in the present study was beneficial for flag leaf length and width parameters of the analysed timothy cultivar, thus increasing assimilative leaf area. These findings correspond with the study by Dromantienė et al. (2015), which showed that different amino acids concentrations used as additional fertilization at the heading stage statistically insignificantly increased winter wheat assimilating leaf area from 2.23 to 20.04 cm^2. A positive effect of amino acids on assimilative leaf area may take some time to show up. Pranckietienė et al. (2009) found that the impact of higher concentrations of amino acids used for spray application on seeds, manifests itself after a longer period of time, i.e. with increasing amino acids concentration the positive effect stands out later (after 28 days). Measurements of chlorophyll index in the leaves showed a significant variation according to the foliar fertilization applied (Table 2). Mean SPAD values during the study years were higher ($P \leq 0.05$) in the plots receiving higher amino acid doses (3.0 and 4.5 L/ha) compared to the control plot. SPAD readings also increased depending on the meas-

Table 1. Comparison of morphological traits of timothy plants depending on the dose of amino acid fertilizer (average for the 3-year study period)

Dose of amino acid fertilizer (L/ha)	Shoot height	Flag leaf		Inflorescence	
		length	width	length	width
Control	113.9 ± 4.05^{c}	5.64 ± 1.02^{bc}	0.38 ± 0.09^{bc}	3.82 ± 0.75^{b}	0.53 ± 0.07^{b}
1.8	116.3 ± 3.22^{bc}	5.84 ± 0.85^{b}	0.41 ± 0.06^{ab}	4.01 ± 0.61^{b}	0.56 ± 0.06^{b}
3.0	117.7 ± 3.51^{b}	6.05 ± 0.62^{ab}	0.43 ± 0.05^{a}	4.26 ± 0.43^{a}	0.56 ± 0.04^{b}
4.5	121.1 ± 2.42^{a}	6.57 ± 0.43^{a}	0.45 ± 0.02^{a}	4.32 ± 0.26^{a}	0.58 ± 0.02^{a}

[a,b,c]Values in columns with different letters differ significantly ($P \leq 0.05$)

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Table 2. Chlorophyll index SPAD in timothy cv. Owacja at different development stages depending on amino acid fertilization (average for the 3-year study period)

Dose of amino acid fertilizer (L/ha)	Stem elongation	Head emergence	Anthesis	Milky ripeness
Control	35.6 ± 1.4[b]	38.7 ± 1.8[b]	40.7 ± 1.1[b]	34.0 ± 1.2[b]
1.8	35.8 ± 1.2[b]	39.4 ± 1.3[b]	41.8 ± 1.4[b]	34.6 ± 1.1[b]
3.0	36.5 ± 0.8[ab]	41.1 ± 1.4[ab]	43.0 ± 0.7[a]	35.8 ± 0.7[ab]
4.5	37.7 ± 0.6[a]	42.9 ± 0.8[a]	44.8 ± 0.5[a]	37.2 ± 0.5[a]

[a,b]Values in columns with different letters differ significantly ($P \leq 0.05$)

urement date and increased up until the flowering stage. Leaf greenness (SPAD) values for the studied plants varied from 35.6 to 44.8 according to the date of measurement. Garcia et al. (2011) indicated that amino acids in nutrient solutions have a favourable effect on mineral leaf status and chlorophyll concentration of tomato leaves. Similarly, Talaat et al. (2005) found that foliar application of tryptophan increased plant growth, photosynthetic pigments (chlorophyll *a*, *b* and carotenoids), soluble and insoluble sugars, total proteins and total alkaloids in the leaves of periwinkle plants (*Catharanthus roseus* (L.) G. Don). Bahari et al. (2013) showed the concentration of photosynthetic pigments in *Triticum aestivum* L. to increase considerably in response to amino acid treatments of seedlings under both normal and salinity stress conditions. Another study evidenced that solutions with different amino acid concentrations, used to fertilize winter wheat at booting, heading and milk maturity stages increased plant photosynthetic indicators. The chlorophyll *a* to *b* ratio in leaves considerably increased when using 0.5–2.5% amino acid solutions at booting and at milk maturity stages. Fertilized plants had a greater assimilative leaf area. Also, Abou Dahab et al. (2006) showed that the chlorophyll content of *Philedendron erubescens* K. Koch & Augustin plants treated with amino acids was higher than in the untreated plants. The seed yield of timothy cv. Owacja varied from 809 (2017) to 1132 kg/ha (2015) (Table 3). As the amino acid fertilizer rate increased, so did the yield of seeds, which peaked when 4.5 L/ha was applied. Successive years of the use also had a significant effect on timothy seed yield, which was the highest during the first year of the study. Moreover, the yield of winter wheat grain increased considerably (0.27–0.4 t/ha) in response to the application of amino acids. The statistical data analysis evidenced that the highest yield and quality would be attained with foliar feeding of winter wheat with amino acid fertilizers: at the booting stage with 2.4%, at the heading stage with 1.47%, and at milk maturity stage with 1.39% amino acids solution (Dromantiene et al. 2015). Table 3 presents the germination capacity of timothy seeds. The value of this parameter depended significantly upon the applied dose of amino acid fertilizer. The highest values ($P \leq 0.05$) were observed in the treatments with the dose of 4.5 L/ha, and slightly lower with the concentration of 3.0 L/ha. Germination capacity was the highest in the first year of seed plantation use

Table 3. Timothy seed yield and germination capacity of timothy seeds depending on the dose of amino acid fertilizer

	Dose of amino acid fertilizer (L/ha)	2015	2016	2017	Mean
Seed yield (kg/ha)	control	994 ± 74[bc]	820 ± 75[c]	809 ± 82[c]	874
	1.8	1003 ± 70[b]	875 ± 64[b]	838 ± 74[b]	905
	3.0	1071 ± 85[a]	942 ± 68[a]	888 ± 84[a]	967
	4.5	1132 ± 62[a]	948 ± 52[a]	915 ± 73[a]	998
Germination capacity (%)	control	90.2 ± 2.41[b]	89.7 ± 2.36[b]	88.4 ± 2.78[b]	89.4
	1.8	92.3 ± 2.34[b]	91.7 ± 2.23[b]	88.7 ± 2.35[b]	90.9
	3.0	93.4 ± 2.17[ab]	92.6 ± 1.98[ab]	89.5 ± 2.13[ab]	91.9
	4.5	95.3 ± 1.92[a]	94.8 ± 1.87[a]	93.5 ± 2.02[a]	94.5

[a,b,c]Values in columns with different letters differ significantly ($P \leq 0.05$)

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and decreased during the subsequent years. Plants developed several internal mechanisms as well as physicochemical factors to ensure that the seeds germinate in favourable environmental conditions. Germination is stimulated by a number of factors including water, temperature, hormonal stimulation, removal of inhibitors and catalytic protein synthesis. Alhadi et al. (2012), who studied the effect of free fatty acid profile on seed germination and seedling development in two cultivars of Yemeni pomegranates, observed changes in amino acid composition of seeds at different germination stages and these changes were thoroughly analysed in the context of their role in dormancy breaking capacities in plant species. It was found that arginin, glutamate and methionine can enhance germination in plants, and so it was assumed that these amino acids regulate germination capacity. Biostimulants, including amino acids, perform a major role in many physiological processes throughout the crop life cycle from seed germination to plant maturity (Calvo et al. 2014).

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