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# EFFECTS OF FOLIAR APPLICATION OF TITANIUM ON SEED YIELD IN TIMOTHY (*Phleum pratense* L.)

### WPŁYW NAWOŻENIA DOLISTNEGO TYTANEM NA PLON NASION W UPRAWIE TYMOTKI ŁĄKOWEJ (Phleum pratense L.)

**Abstract:** Titanium is one of a plant biostimulators. It stimulates life processes, growth and development, as well as affects physiological and biochemical pathways, often increasing biomass production and enhancing yield. An open field experiment was conducted in the years 2011-2013 in Polanowice, Poland to investigate the effects of titanium foliar fertilization on the growth of timothy grass (*Phleum pratense* L.). This single-factor, randomized block design study was performed in four replicates on research plots with the area of 10 m<sup>2</sup> each. The substrate was black loess soil (chernozem) typical for top class farmland. Titanium fertilization *via* leaf spray was performed with a water solution of Tytanit® at three doses of 0.2, 0.4, and 0.8 dm<sup>3</sup>  $\cdot$  ha<sup>-1</sup>. Foliar fertilization with the highest dose of Tytanit® significantly increased seed yield, thousand grain weight and germination capacity. Moreover, the middle dose of Tytanit® (0.4 dm<sup>3</sup>  $\cdot$  ha<sup>-1</sup>) was enough to observe a positive effect on the sample.

Keywords: timothy, titanium fertilizer, seed yield, germination capacity

# Introduction

Timothy (*Phleum pratense* L.), a species of high fodder value, is grown in cold and humid climates. It is one of fodder grasses producing high yield of good quality in terms of green and dry matter, and it also shows good digestibility and palatability. Timothy is a desired component of mixed fodder, and together with red clover, it is typically cultivated in grasslands like meadows or pastures [1]. Fertilization is very important for timothy yield and it may strongly affect biological usability of this grass. Modern agriculture, apart from basic nutrition with mineral fertilizers and application of fungicides and herbicides, widely employs additional supplements such as biostimulators and growth regulators [2]. These substances are applied especially in disadvantageous environments, *eg* in unheated plastic

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tunnels [3, 4]. Such supplements stimulate the growth of a whole plant or its parts, *ie* roots or leaves. Accelerated plant metabolism can increase immunity against stress factors, as well as facilitate regeneration after damage. Biostimulators may also improve germination capacity [5]. They are applied at very low doses referred to as "microfertilization", and allow for limiting the use of other crop protection chemicals [6]. Titanium is one of such supplements used in plant nutrition. Beneficial effects of Ti in agriculture were reported for the first time over 100 years ago in tobacco. Despite numerous reports on positive effects of Ti supplementation, the mechanism of its effectiveness is still unclear [7]. Some attempts were made at elucidating this mechanism by examining Ti-fertilized oats growing in hydroponic and soil conditions [8, 9]. Beneficial effects of Ti fertilization under N malnutrition were investigated by Haghighi in tomato [10]. While Ti fertilization is beneficial, it is not essential and there is no such thing as Ti deficiency in living organisms. Presence of Ti in plant tissues stimulates the activity of such enzymes as nitrate reductase, lipoxygenase, peroxidase, fructose-1,6-bisphosphatase [8, 11]. Plant supplementation with Ti improves their growth and chlorophyll content, pollination, fruit and seed formation [12]. Ti accelerates the growth of leaves, strengthens plants against mildew infection and acts as an antibiotic [13, 14]. Investigations on plant fertilization with Ti are focused mainly on vegetables and crop plants such as rape, winter wheat or sugar beets, and recently also on annual bedding plants [15, 16]. Mitigating effects were observed in Ti-fertilized plants exposed to disadvantageous meteorological circumstances eg low temperature and high precipitation rate that decrease the activity of pollination insects. Ti-triggered improvement was probably due to higher pollen vigour and its better adhesion to the pistil as well as intensive development of the pollen tube [17].

Those results confirm favourable outcomes of Ti fertilization in agriculture [15]. However, there are very few reports concerning the application of Ti fertilizer in grasses [18]. Therefore, the aim of this study was to determine the effects of foliar fertilization with Ti as a growth stimulator on seed yield and seed quality in timothy.

#### Materials and methods

The study was performed in the years 2011-2013 at the Plant Breeding Station in Polanowice (20°09' E, 50°20' N) near Krakow (220 m a.s.l.). The substrate was degraded chernozem derived from loess. Chemical parameters of the soil were as follows:  $pH_{KCl}$  6.8, mineral components: P-55.0, K-125.4, and Mg-46.7 mg  $\cdot$  kg<sup>-1</sup>. Total precipitation ranged from 390 to 631 mm annually over the investigation period, and from 240 to 417 mm annually the growing seasons (Fig. 1). The average annual air temperature was from 4.8 to 6.0°C, and during the growing seasons it was between 10.8 and 12.2°C (Fig. 2).

The experiment followed a randomized block design in four repetitions (plot area:  $10 \text{ m}^2$ ). There were four experimental variants, *ie* control without Ti application and three plots involving Ti foliar fertilization in the form of water solution of Tytanit® at three different concentrations: 0.2, 0.4, and 0.8 dm<sup>3</sup> · ha<sup>-1</sup>.

The experiment followed a randomized block design. Four replicates of four variants were established - control and three doses of Tytanit® (0.2, 0.4 and 0.8 dm<sup>3</sup>  $\cdot$  ha<sup>-1</sup>). In total, 16 plots (1.5 x 6.67 m) were used and the mentioned Tytanit® doses were applied each year in the same manner. The preparation was applied using STIHL backpack sprayer SG 20 (Waiblingen, Germany).

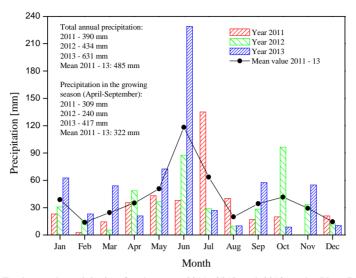


Fig. 1. Total annual precipitation for the years 2011, 2012 and 2013 at the Plant Breeding Station in Polanowice

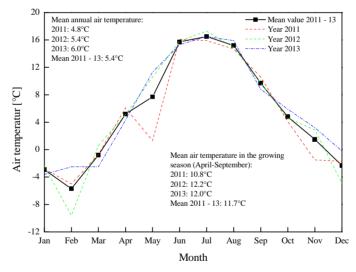


Fig. 2. Annual air temperature for the years 2011, 2012 and 2013 at the Plant Breeding Station in Polanowice

A commercial preparation Tytanit® contains 8.5 g of Ti (in the form of citric acid and ascorbic acid complexes stabilized with potassium sorbate) per 1 dm<sup>3</sup> of a solution, which translates the doses of Tytanit® (0.2, 0.4 and 0.8 dm<sup>3</sup> · ha<sup>-1</sup>) into 1.7, 3.4, and 6.8 g Ti · ha<sup>-1</sup>, respectively. This fertilizer belongs to biostimulators and anti-stress agents approved by the Minister of Agriculture and Rural Development, approval number S-237/11. Tytanit® is produced by INTERMAG LLC, Olkusz, Poland. The first foliar

application was performed at the stem elongation stage, the second two weeks later. The experimental plant was timothy 'Egida' var., registered at the Polish National List of Agricultural Plant Varieties for the Plant Breeding Station Polanowice near Krakow. This variety has been registered in the National Register since 2005 and it is bred at Malopolska Hodowla Roslin (Breeding and Seed Production Station) HPB in Krakow.

Mineral fertilizers were used prior to sowing at the following doses - 50 kg N  $\cdot$  ha<sup>-1</sup> as NH<sub>4</sub>NO<sub>3</sub>, 26.2 kg P  $\cdot$  ha<sup>-1</sup> as Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>, and 66.4 kg K  $\cdot$  ha<sup>-1</sup> as 57% KCl. Additionally, P and K were used each autumn at the same doses and in the same forms as in the spring. Soil nutrition with N was carried out in three batches. The first portion of N 40 kg  $\cdot$  ha<sup>-1</sup> was applied after seed harvest, the second portion of N 20 kg  $\cdot$  ha<sup>-1</sup> was applied in the spring at the beginning of the vegetation (2011 - 21<sup>st</sup> March, 2012 - 26<sup>th</sup> March, 2013 - 12<sup>th</sup> April), and the third portion of N 40 kg  $\cdot$  ha<sup>-1</sup> was administered at the turn of April and May (2011 - 28<sup>th</sup> April, 2012 - 5<sup>th</sup> May, 2013 - 15<sup>th</sup> May).

Timothy seeds were sown at a density of 4 kg  $\cdot$  ha<sup>-1</sup> without any cover crop. Herbicide protection was initiated at the tillering stage in the spring, at all the experimental plots. To remove dicot weeds all plots were treated with Starane 250 SL at a dose of 0.6 dm<sup>3</sup>  $\cdot$  ha<sup>-1</sup> (Dow AgroSciences, Poland). Monocotyledon weeds were eliminated in the beginning of September and in the spring, after the beginning of the vegetation, with Stomp 330 EC solution (BASF, Agro B.V., Wädenswil/Au, Switzerland), made by diluting 5 dm<sup>3</sup>  $\cdot$  ha<sup>-1</sup> of the compound with 300 dm<sup>3</sup> of water. All the plots were preventively sprayed with Decis 2,5 EC (Bayer CropScience S.A., Poland) (0.3 dm<sup>3</sup>  $\cdot$  ha<sup>-1</sup>). The first application took place in the early spring at the beginning of the vegetation, and the second after head emergence but before anthesis 2011 - 21<sup>st</sup> March, 2012 - 26<sup>th</sup> March, 2013 - 12<sup>th</sup> April).

Each year the SPAD index was controlled with the chlorophyll measurement instrument Minolta SPAD 502DL (Minolta, Osaka, Japan). The measurement was performed at the top leaves and included 30 fully developed leaves from each experimental plot (Table 3).

Morphological traits were assessed on generative shoots at anthesis (June). Tiller length, flag leaf length, flag leaf width, inflorescence length and inflorescence width were estimated with a caliper or tape measure (Table 2).

Seed harvest was performed with a combine harvester Wintersteiger NM-ELITE (Wintersteiger AG Ried, Austria) at the turn of July and August. After the harvest, the plots were cleaned from straw and plant debris. The seeds were separated, cleaned and dried up to 14% water content. Kernel weight and germination capacity were determined for each experimental plot after seed cleaning. Germination capacity was calculated at the 10<sup>th</sup> day of germination in a Jacobsen germinator (Laborset, Poland) according to Polish standard PN-79/R-65950.

The results were subjected to the analysis of variance and verified by means of Duncan's test with Statistica 10 PL software.

#### Results

*Development stages.* Accelerated plant aging was observed following Ti fertilization (Table 1). For example, head emergence stage was reached 76 days after the application of Ti at 1.7 g  $\cdot$  ha<sup>-1</sup>, 75 days after spraying the plants with Ti at 3.4 - 6.8 g  $\cdot$  ha<sup>-1</sup>, and after 78 days in control plants. Anthesis occurred 91 days after the application of Ti at

1.7 g  $\cdot$  ha<sup>-1</sup>, 90 days after spraying the plants with Ti at 3.4 - 6.8 g  $\cdot$  ha<sup>-1</sup>, and after 92 days in control plants. Achieving maturity took 127 days in the plants sprayed with Ti at 1.7 g  $\cdot$  ha<sup>-1</sup>, 126 days in the plants treated with Ti at 3.4 - 6.8 g  $\cdot$  ha<sup>-1</sup> and 129 days in the control plants.

Table 1

| $Tytanit  dose [dm3 \cdot ha-1]$ | Head emergence            | Anthesis                  | Maturity                   |
|----------------------------------|---------------------------|---------------------------|----------------------------|
| 0 (control)                      | 78.0 (±1.0) <sup>a</sup>  | 92.0 (±1.0) <sup>a</sup>  | 128.7 (±0.6) <sup>a</sup>  |
| 0.2                              | 76.3 (±0.6) <sup>ab</sup> | 91.3 (±1.5) <sup>ab</sup> | 127.3 (±1.2) <sup>ab</sup> |
| 0.4                              | 75.3 (±2.3) <sup>b</sup>  | 90.3 (±0.6) <sup>b</sup>  | 126.3 (±1.5) <sup>b</sup>  |
| 0.8                              | 74.7 (±1.2) <sup>b</sup>  | $89.7 (\pm 2.5)^{b}$      | 125.7 (±0.6) <sup>b</sup>  |

Duration of main development stages of timothy (days), depending on Ti dose. Mean values based on three years of observations (2011-2013)

a, b - different letters in columns denote statistically significant differences ( $P \le 0.01$ )

*Morphology and chlorophyll content.* The changes in morphological parameters measured over three years of the experiment indicated that Ti application encouraged stem growth. Ti-fertilized timothy formed stronger stems with longer tillers, longer and broader leaves and longer and broader inflorescences (Table 2). Ti dose of 6.8 g  $\cdot$  ha<sup>-1</sup> increased the tiller length by 5%, flag leaf length by 51%, flag leaf width by 55%, inflorescence length by 51% and inflorescence width by 23%, as compared with the control plants.

Table 2

| Tytanit® dose<br>[dm <sup>3</sup> · ha <sup>-1</sup> ] | Tiller length              | Flag leaf length         | Flag leaf width            | Inflorescence<br>length | Inflorescence<br>width     |
|--|----------------------------|--------------------------|----------------------------|-------------------------|----------------------------|
| 0 (control)  | 113.4 (±2.0) <sup>b</sup>  | 5.3 (±0.5) <sup>b</sup>  | 0.40 (±0.05) <sup>b</sup>  | 5.4 (±1.6) <sup>c</sup> | 0.52 (±0.05) <sup>b</sup>  |
| 0.2  | 115.8 (±3.1) <sup>b</sup>  | 7.4 (±0.5) <sup>ab</sup> | $0.46 (\pm 0.09)^{ab}$     | $5.7 (\pm 1.0)^{bc}$    | $0.58 (\pm 0.08)^{ab}$     |
| 0.4  | 117.2 (±2.2) <sup>ab</sup> | 6.6 (±1.1) <sup>ab</sup> | 0.56 (±0.07) <sup>ab</sup> | 6.6 (±1.8) <sup>b</sup> | 0.62 (±0.04) <sup>ab</sup> |
| 0.8  | 119.6 (±2.1) <sup>a</sup>  | 8.0 (±1.0) <sup>a</sup>  | 0.62 (±0.19) <sup>a</sup>  | 8.2 (±0.6) <sup>a</sup> | 0.64 (±0.04) <sup>a</sup>  |
| Standard dev.  | 2.6                        | 1.2                      | 0.1                        | 1.3                     | 0.05                       |
| Variation coeff. (%)                                   | 2.2                        | 17.1                     | 19.3                       | 19.3                    | 9.0                        |

Differences in morphological parameters depending on Ti dose [cm]

a, b, c - different letters in columns denote statistically significant differences ( $P \le 0.05$ )

Foliar fertilization with Ti considerably increased chlorophyll concentration in timothy leaves. This effect was particularly pronounced at the stage of anthesis (Table 3). The average values of chlorophyll index SPAD were always higher ( $P \le 0.05$ ) in Ti treated plants than in the controls. Even the lowest applied dose of Ti, *ie* 1.7 g  $\cdot$  ha<sup>-1</sup> produced statistically meaningful effects. The application of Ti at a dose of 3.4 g  $\cdot$  ha<sup>-1</sup> increased leaf chlorophyll index SPAD by about 13% at the stage of anthesis. Further supplementation with Ti at a dose of 6.8 g  $\cdot$  ha<sup>-1</sup> caused 17% increase in the SPAD, indicating that a saturation effect of fertilization with Ti can be reached by applying Ti at a dose of *ca* 7 g  $\cdot$  ha<sup>-1</sup>.

Table 3

| Tytanit® dose             | Development stage        |                          |                          |                          |  |
|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--|
| $[dm^3 \cdot ha^{-1}]$    | Stem elongation          | Head emergence           | Anthesis                 | Milky Ripeness           |  |
| 0 (control)               | 36.5 (±1.6) <sup>b</sup> | 37.8 (±0.9) <sup>b</sup> | 38.6 (±1.7) <sup>b</sup> | 35.5 (±1.5) <sup>b</sup> |  |
| 0.2                       | 37.1 (±1.4) <sup>b</sup> | 38.5 (±1.4) <sup>b</sup> | 41.8 (±0.7) <sup>b</sup> | 37.2 (±0.5) <sup>b</sup> |  |
| 0.4                       | 38.4 (±0.8) <sup>a</sup> | 40.8 (±0.5) <sup>a</sup> | 43.9 (±0.8) <sup>a</sup> | 38.4 (±0.5) <sup>a</sup> |  |
| 0.8                       | 39.5 (±0.5) <sup>a</sup> | 42.2 (±0.5) <sup>a</sup> | 45.8 (±0.9) <sup>a</sup> | 40.3 (±0.7) <sup>a</sup> |  |
| Mean value                | 37.9                     | 39.8                     | 42.5                     | 37.9                     |  |
| Standard deviation        | 1.3                      | 2.0                      | 3.1                      | 2.0                      |  |
| Variation coefficient [%] | 3.5                      | 5.1                      | 7.3                      | 5.3                      |  |

Chlorophyll index SPAD in timothy grass variation 'Egida' at different development stages depending on Ti fertilization (mean values from three years of the study)

a, b - different letters in columns denote statistically significant differences ( $P \le 0.05$ )

Seed yield. Timothy seed yield ranged from 117.3 g  $\cdot$  m<sup>-2</sup> in 2011 to 68.1 g  $\cdot$  m<sup>-2</sup> in 2013 (Table 4). Seed yield always increased following Ti application. Ti at a dose of 3.4 g  $\cdot$  ha<sup>-1</sup> increased the seed yield by about 17% (mean value for three years of the study). Fertilization with 6.8 g  $\cdot$  ha<sup>-1</sup> still increased the seed yield, however, the saturation effect appeared. Seed yield in this variant was by 19% higher (mean value for three years of the study) as compared to the control.

Table 4

| Tytanit® dose             |                            | Mean value                 |                          |             |
|---------------------------|----------------------------|----------------------------|--------------------------|-------------|
| $[dm^3 \cdot ha^{-1}]$    | 2011                       | 2012                       | 2013                     | Wiean value |
| 0 (control)               | 98.2 (±1.8) <sup>b</sup>   | 87.2 (±2.8) <sup>b</sup>   | 68.1 (±1.4) <sup>b</sup> | 84.5        |
| 0.2                       | 107.2 (±2.6) <sup>ab</sup> | 100.2 (±2.7) <sup>ab</sup> | 70.9 (±1.6) <sup>b</sup> | 92.8        |
| 0.4                       | 116.3 (±1.9) <sup>a</sup>  | 107.2 (±1.9) <sup>a</sup>  | 73.9 (±1.7) <sup>a</sup> | 99.1        |
| 0.8                       | 117.3 (±2.8) <sup>a</sup>  | 109.2 (±1.9) <sup>a</sup>  | 76.6 (±1.8) <sup>a</sup> | 101.0       |
| Mean value                | 109.8                      | 101.0                      | 72.4                     | -           |
| Standard deviation        | 8.9                        | 10.0                       | 3.7                      | -           |
| Variation coefficient [%] | 8.2                        | 9.9                        | 5.1                      | -           |

Timothy seed yield as a function of Ti fertilization  $[g \cdot m^{-2}]$ 

a, b - different letters in columns denote statistically significant differences ( $P \le 0.05$ )

Seed weight. Timothy thousand grain weight (g) increased as a result of Ti fertilization (Table 5). Considerable improvement was observed already at Ti dose of  $3.4 \text{ g} \cdot \text{ha}^{-1}$ . In this case, timothy thousand grain weight was higher by about 30% than in the control plants. Similarly to other observations, the saturation effect of fertilization could be detected for Ti dose of 6.8 g  $\cdot$  ha<sup>-1</sup>. At this Ti concentration, mean difference between Ti treatment and control plants for the entire study period was 33%.

*Germination capacity*. Table 6 represents germination capacity of the seeds obtained from Ti-fertilized plants as a function of Ti dose. This parameter increased when Ti supplementation was applied. Explicit enhancement was observed at Ti dose of  $3.4 \text{ g} \cdot \text{ha}^{-1}$ , with 6% difference between Ti-treated and control plants over the entire study period. Saturation effect was reached at Ti dose of  $6.8 \text{ g} \cdot \text{ha}^{-1}$ , and average 3-year difference between this variant and control was 7%.

| Tytanit® dose             |                             | Mean value                  |                             |             |
|---------------------------|-----------------------------|-----------------------------|-----------------------------|-------------|
| $[dm^3 \cdot ha^{-1}]$    | 2011                        | 2012                        | 2013                        | wiean value |
| 0 (control)               | 0.431 (±0.017) <sup>b</sup> | 0.421 (±0.014) <sup>b</sup> | 0.400 (±0.006) <sup>b</sup> | 0.417       |
| 0.2                       | 0.471 (±0.023) <sup>b</sup> | 0.461 (±0.018) <sup>b</sup> | 0.431 (±0.008) <sup>b</sup> | 0.454       |
| 0.4                       | 0.561 (±0.016) <sup>a</sup> | 0.541 (±0.025) <sup>a</sup> | 0.521 (±0.012) <sup>a</sup> | 0.541       |
| 0.8                       | 0.571 (±0.014) <sup>a</sup> | 0.561 (±0.022) <sup>a</sup> | 0.531 (±0.017) <sup>a</sup> | 0.554       |
| Mean value                | 0.509                       | 0.496                       | 0.471                       | -           |
| Standard deviation        | 0.068                       | 0.066                       | 0.065                       | -           |
| Variation coefficient [%] | 13.5                        | 13.3                        | 13.8                        | -           |

Timothy thousand grain weight [g] depending on Ti dose

a, b - different letters in columns denote statistically significant differences ( $P \le 0.05$ )

Table 6

| Timothy seed | germination | capacity [% | ] deper | nding o | on Ti c | lose |
|--------------|-------------|-------------|---------|---------|---------|------|
|--------------|-------------|-------------|---------|---------|---------|------|

| Tytanit® dose             |                          | Mean value               |                          |             |
|---------------------------|--------------------------|--------------------------|--------------------------|-------------|
| $[dm^3 \cdot ha^{-1}]$    | 2011                     | 2012                     | 2013                     | wiean value |
| 0 (control)               | 88.3 (±2.5) <sup>b</sup> | 86.7 (±2.6) <sup>b</sup> | 85.4 (±1.8) <sup>b</sup> | 86.8        |
| 0.2                       | 89.7 (±1.6) <sup>b</sup> | 88.8 (±1.8) <sup>b</sup> | 88.3 (±1.6) <sup>b</sup> | 88.9        |
| 0.4                       | 93.7 (±2.0) <sup>a</sup> | 92.4 (±1.4) <sup>a</sup> | 90.2 (±1.2) <sup>a</sup> | 92.1        |
| 0.8                       | 96.3 (±1.4) <sup>a</sup> | 92.9 (±1.7) <sup>a</sup> | 90.7 (±2.4) <sup>a</sup> | 93.3        |
| Mean value                | 92.0                     | 90.2                     | 88.7                     | -           |
| Standard deviation        | 3.7                      | 3.0                      | 2.4                      | -           |
| Variation coefficient [%] | 4.0                      | 3.3                      | 2.7                      | -           |

a, b - different letters in columns denote statistically significant differences ( $P \le 0.05$ )

#### Discussion

The study results indicated that Ti applications significantly affected morphological parameters, chlorophyll index, seed yield, and seed germination in timothy. A comparison of mean values demonstrated that foliar Ti application considerably improved all investigated morphological parameters such as tiller length, flag leaf length, flag leaf width, inflorescence length, and inflorescence width. The shortest plants were harvested from the control plots, whereas the highest from the plots treated with Ti at a dose of 6.8 g  $\cdot$  ha<sup>-1</sup>. Contrasting results were obtained by Jaberzadeh et al [19] and Whitted-Haag et al [16]. Jaberzadeh et al [19] reported that higher concentration of Ti (0.03%) in *Triticum aestivum* contributed to decreased plant height and improved biomass yield and seed parameters. According to Whitted-Haag et al [16] Ti supplementation (at 0, 25, 50, 75, and 100 mg · dm<sup>-3</sup>) in annual bedding plants (geranium, impatiens, pansy, petunia, and snapdragon) only slightly affected plant height but significantly changed stem diameter. In Impatiens walleriana stem diameter exhibited a linear decrease as Ti concentration increased (from 8.9 mm in control to 7.4 mm in the plants treated with Ti at 100 mg  $\cdot$  dm<sup>-3</sup>). In addition, Ti supplementation markedly decreased the number of leaves per plant in I. walleriana, from 31 leaves in the control plants to 27 leaves in plants fertilized with Ti at 100 mg  $\cdot$  dm<sup>-3</sup>. The outcomes presented by Whitted-Haag et al [16] demonstrated significant negative effect of Ti supplementation on chlorophyll SPAD content in the leaves of annual bedding plants. Ti supplementation linearly decreased chlorophyll SPAD index in Impatiens walleriana (chlorophyll content decreased from 36.8 in control to 33.2 mg  $\cdot$  m<sup>-2</sup> in plants treated with the highest dose of Ti, *ie* 100 mg  $\cdot$  dm<sup>-3</sup>). Our results do not confirm

Table 5

these reports. This study showed that formation of stronger shoots with longer tillers, longer and broader leaves was due to increased chlorophyll content in the leaves. Similar results were obtained by Radkowski [18], who found that foliar fertilization of timothy with Ti at  $6.8 \text{ g} \cdot \text{ha}^{-1}$  resulted in an increase in SPAD value. Additionally, the plants treated with the lowest dose of Ti (1.7 g · ha<sup>-1</sup>) exhibited ca. 2% lower chlorophyll content than the control ones. In this study, foliar fertilization with Ti significantly increased chlorophyll content in timothy leaves. The application of Ti ( $3.4 \text{ g} \cdot \text{ha}^{-1}$ ) increased the chlorophyll index SPAD by 13% at the anthesis stage. Raskar and Lawares [20] indicated that low Ti concentrations might promote seed germination and early seedling growth in onion, while high concentrations of Ti ( $10 \text{ to } 50 \ \mu\text{g} \cdot \text{cm}^{-3}$ ) in onion and claimed that titanium applied in low concentration ( $30 \text{ to } 40 \ \mu\text{g} \cdot \text{cm}^{-3}$ ) enhanced seed germination, promptness index and seedling growth. Our study partly confirmed these results, as Ti application significantly improved timothy seed germination.

Small doses of Ti positively affected timothy growth rate. Increasing dose of Ti caused the saturation effect in the tissues, which in the case of foliar application in timothy was achieved at a dose of Ti ca. 7 g  $\cdot$  ha<sup>-1</sup> (applied twice). Results published by other researches showed that high doses of Ti decreased plant growth. Therefore, we claim the optimal dose of Ti may be different for different species and may depend on the application method.

Seed crop yield usually decreases in the second and third year of cultivation and this was also observed in our study. Similar observation was reported by other authors [22]. Nowadays, seed crops in the UK are grown for 1-2 years and then they are renewed. Disadvantageous conditions for seed yield include strong thermal and humidity fluctuations during the growth period. Optimal temperature range at the stage of anthesis and seed formation in *Phleum pratense* is 15-18°C. In our study, such thermal conditions occurred each year of the experiment, meaning that thermal conditions were favourable in all years of the study. However, increased precipitation in June 2013 was probably one of the reasons for lower seed yield that year. High precipitation at anthesis and ripening lowers the seed yield.

An increase in timothy yield up to 20%, higher seed yield and grain weight resulting from microfertilization with Ti at a dose of a few grams per hectare may be explained neither by simple nutrition effects nor by micronutrition as in the case of Mg, Fe, Mn, or Zn. Such small amounts of Ti could only affect the yield if this element acted as a catalyst. The biochemical processes affected by Ti are still not fully understood and are actually a subject of speculation. A hypothesis put forward by Cigler et al [23] assumes that Ti affects photosynthetic processes. However, the primary effect of fertilization with Ti manifested as an increase in leaf chlorophyll concentration (SPAD index exceeding 20%), gives timothy the initial gain in the growth. One of the consequences is a formation of stronger shoots with longer tillers and longer and broader leaves. Finally, strong shoots with high concentrations of chlorophyll produce more glucose, which translates into faster synthesis of biopolymers such as cellulose or starch in the seeds. This can be understood as a secondary effect of Ti fertilization. Stronger plant shoots allow for the formation of longer and broader inflorescences. All these processes are accelerated by Ti fertilization. Finally, stronger plants produce bigger and healthier seed kernels. In 2002, Janas reported higher yield (by about 50%) of Ti-fertilized aubergine despite disadvantageous climate conditions [3]. Dobromilska [24] observed stimulating effects of Ti in tomatoes. These observations were explained by higher intracellular activity of iron ions and thus accelerated synthesis of photosynthetic pigments. Intensification of metabolism and other growth processes like pollination, fruit and seed formation in maize were also observed by Ertani [17]. Kuzel et al [8] proposed to describe the effects of Ti as "hormosis". The impact of Ti fertilization is usually weaker in the case of foliar application than when it is provided together with other nutritional agents in a hydroculture. The experiments with oat (*Avena sativa* L. cv. Zlatak) grown in two different soils and fertilized with three different concentrations of Ti solution showed strong influence of Ti on all measured parameters. The observed effects were independent of soil type [8]. Tlustos et al [15] investigated the impact of combined foliar application of Ti and Mg on the yield and content of fodder ingredients in potato, wheat and barley. Studies involved full fertilization with N and its deficiencies. N fertilization strongly affected the results of foliar fertilization with Ti. A study on nutrition in tomatoes grown in rockwool showed improved yield and a simultaneous increase in the content of N, P, Ca, Mg and K in the plants fertilized with Ti (at 24.2 mg Ti per plant year<sup>-1</sup>) [25].

All these observations seem to justify the conclusion that Ti may improve plant enzyme activities, particularly those involved in the photosynthesis redox processes. It may be also engaged in the absorption of nutrients from the soil by generating lower osmotic pressure in the above ground parts of plants. Plants respond to decreased osmotic pressure in the above ground parts by accelerated absorption of water by the root system and consequently higher intake of nutrients from the soil.

#### Conclusions

Ti foliar microfertilization at a dose of 3.4 and 6.8 g  $\cdot$  ha<sup>-1</sup> improves the yield of timothy by about 17-20% with reference to seed yield, seed kernel weight and germination capacity. At a dose of 6.8 g Ti  $\cdot$  ha<sup>-1</sup> (two applications) the tissue saturation effect is achieved. This beneficial effect seems to be due to the acceleration of photosynthesis redox processes. A simple economic feasibility analysis shows profitability of Ti fertilization (at a dose of 3.4-6.8 g  $\cdot$  ha<sup>-1</sup>) in timothy grass crop in the amount of 156-170 \$ per 1 ha of arable land (prices calculated for Krakow region taking into account the cost of Tytanit® and its application). The application of Ti in agriculture is a sustainable intensification method for raising productivity. We assume that similar effects can be achieved with other grass or grain species. Optimal dose of Ti may be different for different species and may depend on the application method.

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#### WPŁYW NAWOŻENIA DOLISTNEGO TYTANEM NA PLON NASION W UPRAWIE TYMOTKI ŁĄKOWEJ (Phleum pratense L.)

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**Abstrakt:** Doświadczenie prowadzono w latach 2011-2013 na terenie Stacji Hodowli Roślin w Polanowicach pod Krakowem (220 m n.p.m.). Celem eksperymentu było zbadanie wpływu tytanu (Ti) na plon i jakość nasion tymotki łąkowej odmiany 'Egida'. Jednoczynnikowe doświadczenie polowe założono metodą losowanych bloków, w czterech powtórzeniach, powierzchnia poletek doświadczalnych wynosiła 10 m<sup>2</sup>. Na polu doświadczalnym występował czarnoziem zdegradowany wytworzony z lessu, zaliczany pod względem bonitacyjnym do klasy I. Czynnikiem doświadczenia był oprysk biostymulatorem wzrostu w postaci nawozu Tytanit® w trzech dawkach: 0,2, 0,4 i 0,8 dm<sup>3</sup>  $\cdot$  ha<sup>-1</sup>. Zastosowanie nawożenia dolistnego Tytanitem® w najwyższej dawce (0,8 dm<sup>3</sup>  $\cdot$  ha<sup>-1</sup>) spowodowało istotny wzrost plonów nasion, masy tysiąca nasion oraz zdolności kiełkowania w stosunku do obiektu kontrolnego. Zadowalające efekty uzyskano już w obiektach, gdzie aplikowano Tytanit® w dawce 0,4 dm<sup>3</sup>  $\cdot$  ha<sup>-1</sup>.

Słowa kluczowe: tymotka łąkowa 'Egida', biostymulator wzrostu, masa tysiąca nasion, zdolność kiełkowania