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Original article

The effect of growth regulator Tytanit dose on *Medicago* x *varia* T. Martin and *Trifolium pratense* L. yield and nutritional value

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ABSTRACT

The aim of the research was to evaluate the effect of foliar application of different doses of Tytanit as a biostimulant on the yield and nutritional value of *Medicago* × *varia* T. Martin and *Trifolium pratense* L. It was assumed that titanium application during any life cycle of alfalfa hybrid and red clover would contribute to their growth, digestibility, and total protein content. In addition, it was expected that increasing doses of Tytanit up to 0.6 dm^3 ha^{-1} would improve the yield and quality of forage plants. Different doses of Tytanit in different ways affected the yield. However, the largest dose of 0.6 dm^3 turned out to be the most effective. It contributed to a 38% increase in the yield of hybrid alfalfa and to a 31% increase in the red clover yield. Individual doses of Tytanit in different ways affected accumulation of protein and crude fibre in the dry matter. Used at 0.4 and 0.6 dm^3 doses it increased the amounts of protein and crude fibre relative to control. The smallest dose had no significant effect on these parameters. Tytanit did not improve dry matter digestibility, and there was no statistically significant variation as a result of its application. Foliar application of the biostimulant resulted in an increase in the concentration of phosphorus, potassium, and magnesium in plant dry matter. High content of calcium in the plant species before Tytanit application increased further as a response to 0.2 and 0.4 dm^3 doses, with a slight increase in the ratio of K: (Ca + Mg) and an excessive growth of the Ca: P ratio, which reduced hybrid alfalfa and red clover nutritional values. Thus, Tytanit doses used in the experiment significantly increased hybrid alfalfa and red clover yields, but the nutritional value of the plants did not improve.

1. Introduction

The widespread use of chemical plant protection products and fertilisers is one of the causes of environmental degradation (Grajkowski and Ochmian, 2007). The solution for this state of affairs is integrated farming, which can reduce use of chemical products in plant-growing technologies, replacing them with biostimulants and other substances. Therefore, new effective methods and environmentally friendly solutions are needed to improve the health and quality of plants (Ilieva and Vasileva, 2013; Janas et al., 2013; Serrano et al., 2004; Szparaga et al., 2018; Kocira et al., 2019; Vasileva et al., 2017). One of such biostimulants is Tytanit (Godlewska and Ciepiela, 2018; Malinowska and Kalembasa, 2012; Radkowski and Radkowska, 2013; Radkowski et al., 2015; Wadas and Kalinowski, 2017a, b), the active ingredient of which is titanium (Ti). This chemical element is a micronutrient having a positive effect on plant biochemistry, supporting processes leading to an acceleration of plant growth and to an increase in the production of biomass (Kužel et al., 2003; Lyu et al., 2017; Radkowski and Radkowska, 2010). Substances containing titanium stimulate the development of the whole plant or its individual parts by increasing the dynamics of life processes (Yildirim et al., 2002). According to the manufacturer (Cieśliński, INTERMAG) Tytanit has been produced in Poland since 1989. Initially it was qualified as mineral fertiliser, but since 2011 it has been classified as mineral growth regulator. In its composition it contains 8.5 g of titanium per 1 dm^3 (Cieśliński, INTERMAG). The way of action makes Tytanit and other regulators safe for the environment, and their use helps to reduce the amount of chemicals used in agriculture (Ertani et al., 2009; Maini, 2006).

As one of trace elements titanium plays a very important role in plant physiology. The literature confirms that it is a plant growth

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regulator (Kleiber and Markiewicz, 2013; Michalski, 2008). The role of this type of substances (regulators) is to reduce the negative impact of adverse environmental conditions on plants, such as unfavourable air humidity, air temperature, and light intensity, or poor soil quality with inadequate access to nutrients (Grenda, 2003; Wójcik and Wójcik, 2001). Regulators reduce water and air humidity requirements, increase resistance to low temperatures, but also accelerate regeneration after plant damage and increase resistance to fungal diseases and bacterial infections (Marcinek and Hetman, 2007). In plants, titanium stimulates activity of such enzymes as catalase, peroxidase, lipoxygenase, or nitrate reductase (Ertani et al., 2009), and it also increases chlorophyll content in leaves (Dobromilska, 2007). According to Michalski (2008) titanium has a beneficial effect on iron ions, increasing their activity, and on pollen vigour; it improves plant health and intensity of nutrient uptake. It can have a significant impact on the quality of plant raw material, for example, on the content of ascorbic acid or calcium (Brown and Saa, 2015; Skupień and Oszmiański, 2007). As mentioned above, titanium, used together with biostimulants containing hormones, protein, or other trace elements, increases the activity of iron ions, which is positively correlated with the intensity of photosynthesis, chlorophyll synthesis, and action of the enzymes responsible for neutralizing free radicals. All these processes are conducive to the growth and development of plants, improving their health and resistance to stress caused by low temperature or limited access to light (Skupień and Oszmiański, 2007). In addition, titanium limits plant damage caused by heavy metals (Leskó et al., 2002). It is one of the elements with the lowest phytoaccumulation index, with the exception of plants absorbing a large amount of silicon (Malinowska and Kalembasa, 2012). According to Tlustoš et al. (2005) the effect of titanium foliar application largely depends on nitrogen content in the plants. Response of plants after its application declines with growing nitrogen deficiency. In turn, Kužel et al. (2007) report that titanium effect can be increased by simultaneous magnesium application.

Being common in nature titanium is the tenth most abundant element building the Earth's lithosphere, with its content in soil ranging from 0.1 to 0.5% (Gworek, 1990). It is present in such minerals as anatase, rutile, and brookite, which consist in 95% of TiO^2, and in ilmenite with about 40–65% of TiO^2. These minerals are generally insoluble in water, therefore, titanium is generally considered to be indifferent to environmental pollution (Lyu et al., 2017). Gworek (1990) found that brown soil contained substantial amounts of titanium (0.387%), with luvisol soil containing the least (0.080%). The distribution of this chemical element in the soil is dependent mainly on geological processes and not on soil formation processes. In addition, there is a close relationship between the content of fine particles in soil and the content of titanium. With an increase in the content of particles with the diameter of less than 0.02 mm, the percentage of titanium in the soil increases (Gworek, 1990). When present in aqueous solution in available forms this element is beneficial for plant nutrition (Du et al., 2010; Hrubý et al., 2002).

The aim of the research was to evaluate the effect of foliar application of different doses of Tytanit as a biostimulant on the yield and nutritional value of *Medicago × varia* T. Martin and *Trifolium pratense* L. It was assumed that Tytanit application during any life cycle of alfalfa hybrid and red clover would contribute to their growth, digestibility, and total protein content. In addition, it was expected that increasing doses of Tytanit up to 0.6 $dm^3\ ha^{-1}$ would improve the yield and quality of forage plants. The product was expected to have a positive effect on the content of calcium, magnesium, potassium, and phosphorus, regulating their mutual relations in the dry matter of the plant species.

2. Material and methods

2.1. Experimental conditions and experiment location

The experiment was conducted at the experimental facility in Poland (52°10′03″N; 22°17′24″E), between 2015 and 2017. The experiment was carried out in a split-plot design with three replications. The soil consisting of loose loamy sand (Table 1) was recognized as Technosols according to the FAO classification.

Table 2 presents detailed information on the chemical composition of the soil. The C:N ratio was 10.4:1. The pH was close to neutral. In addition, the soil had high content of absorbable forms of P and Mg, but absorbable forms of K were within the limits of the medium amount.

Experimental factors:

The following experimental factors were included in the experiment:

Factor I – plant species:

- hybrid alfalfa (*Medicago × varia* T. Martin) var. Comet,
- red clover (*Trifolium pratense* L.) var. Krynia.

Factor II – the biostimulant (Tytanit) doses:

- $Ti_{0.0}$ - control with distilled water,
- $Ti_{0.2}$–0.2 $dm^3\ ha^{-1}$ (1.2 $dm^3\ ha^{-1}$ annually),
- $Ti_{0.4}$–0.4 $dm^3\ ha^{-1}$ (2.4 $dm^3\ ha^{-1}$ annually),
- $Ti_{0.6}$–0.6 $dm^3\ ha^{-1}$ (3.6 $dm^3\ ha^{-1}$ annually).

During each of the three growth cycles of the plants were treated twice (dividing the annual dose), in the growth and development stages according to the European BBCh scales, separately for alfalfa hybrid and red clover:

- alfalfa hybrid - first application (F1) was during the first nod stage (BBCh 31), and the second (F2) when the first flower buds were visible outside leaves (BBCh 51),
- red clover - the first application (F1) after forming the first lateral branch (BBCh 21), and the second (F2) when the first petals were visible ((BBCh 51).

The spray liquid to be applied to plants was made by diluting individual doses of Tytanit in water (200 $dm^3\ ha^{-1}$), with control units treated with the same amount of water only (Table 3).

2.2. Laboratory analysis

2.2.1. Soil material

Before the sowing samples of the soil were collected to determine its physical and chemical properties. The weight of the soil samples ranged from 0.8 to 1 kg. The samples were subjected to air drying at a temperature of 105 °C. Then soil larger particles were separated by sieving the material through a mesh with a diameter of 2 mm. In the soil samples processed this way the following parameters were determined:

- pH in 0.02 $mol\ dm^{-3}$ $CaCl_2$ solution, determined by potentiometry;
- content of carbon in organic compounds using the oxidation method with titration;
- content of total nitrogen with the Kiejdahl method;
- content of selected nutrients with the AAS method using the Varian SpectrAA-20 spectrophotometer with Merck standards;

Table 1
Soil texture.

Percentage of particles (diameter in mm)						
1–0.1	0.1–0.05	0.05–0.02	0.02–0.06	0.06–0.002	<0.002	Soil type
76	9	5	4	4	2	Loose loamy sand

Table 2
Soil chemical composition.

Nutrient	Content
pH	6.80
C_{org} (g kg^{-1} DM)	13.50
All forms (g kg^{-1} DM)	
N_{og}	1.30
Ca	1.80
Na	0.15
All forms (mg kg^{-1} DM)	
Fe	4562.80
Mn	156.20
Cu	5.60
Zn	14.50
Available forms (mg kg^{-1} DM)	
P - $H_2PO_4^-$	170.00
K - K^+	114.00
Mg - Mg^{2+}	84.00

- content of available forms of phosphorus with the spectrophotometric method;
- content of available forms of potassium with the spectrophotometric method;
- content of available magnesium with flame atomic absorption spectrometry (FAAS).
- soil granulometric composition with fractions, subfractions, groups and granulometric subgroups with the Bouyoucos-Casagrande aerometric method

2.2.2. Plant material

Details of establishing the plantation: *Dactylis glomerata* L., cultivated for two years, was as a forecrop. The seeds were planted in 15 September 2015, rows every 30 cm at an approximate spacing of 4 cm, with the sowing rate complying with the standards for the species (alfalfa hybrid 12 kg ha-1, red clover 11 kg ha-1). The plants reached full development in the growing periods of 2016 and 2017. Weeds were removed mechanically and manually. Chemical plant protection was not required and the seeds were not treated. The experimental plots had an area of 6 m^2 (2 m × 3 m), with a 1 m wide herbicide path between them.

In the growing seasons of the full use of plants three harvests of hybrid alfalfa and red clover were collected (at the time of 30–40% of inflorescences flowering). The dates of harvests are presented in Table 4. At that time the fresh matter form each plot was weighed, and samples of plant material (an average of 1.2 kg) were collected for chemical analysis. The dry matter of plants was specified with the oven-drying method. The plant material subjected to chemical analysis was obtained by grinding leaves, stems, and inflorescences. The dry weight of the plants was determined at the temperature of 105 °C ± 2 °C, drying for 3 h and cooling down for about 1 h.

To assess plant nutritional value the following parameters were determined:

- dry matter yield (Mg ha^{-1}),
- protein and crude fibre content in plant dry matter (g kg^{-1}) with the NIRS method using the N-NIRFlex 500,
- dry matter digestibility (%) with the NIRS method using the N-NIRFlex 500,
- total content (g kg^{-1}) of selected macronutrients (P, K, Ca, and Mg) in plant dry matter with the ICP-AES method,
- the ratio of macronutrients in plant dry matter: K: (Ca + Mg) and Ca: P.

2.3. Weather conditions

Sielianinov's hydrothermal coefficient was calculated in order to determine temporal variation of meteorological conditions and their effects on vegetation. The hydrothermal coefficient (K) was calculated on the basis of monthly sums of precipitation (P) and a sum of monthly air temperatures (t), using the following formula (Skowera and Puła, 2004):

$$K = \frac{P}{0.1}\sum t$$

Table 3
Methods of treatment.

Species	Units	Treatment	Growth and development stages – treatment forms	
			F1[a]	F2[b]
Hybrid alfalfa	$Ti_{0.0}$	200 dm^3 of water	water	water
	$Ti_{0.2}$	0.2 dm^3 ha^{-1} Tytanit + 200 dm^3 of water	spray liquid	spray liquid
	$Ti_{0.4}$	0.4 dm^3 ha^{-1} Tytanit + 200 dm^3 of water	spray liquid	spray liquid
	$Ti_{0.6}$	0.6 dm^3 ha^{-1} Tytanit + 200 dm^3 of water	spray liquid	spray liquid
Red clover	$Ti_{0.0}$	200 dm^3 of water	water	water
	$Ti_{0.2}$	0.2 dm^3 ha^{-1} Tytanit + 200 dm^3 of water	spray liquid	spray liquid
	$Ti_{0.4}$	0.4 dm^3 ha^{-1} Tytanit + 200 dm^3 of water	spray liquid	spray liquid
	$Ti_{0.6}$	0.6 dm^3 ha^{-1} Tytanit + 200 dm^3 of water	spray liquid	spray liquid

[a] F1 – alfalfa: when the first node was visible, red clover: when the first side shoots were visible.
[b] F2 – alfalfa: when the first flower buds were visible outside leaves, red clover: when the first flower buds were visible.

Table 4
Harvest time.

Harvest	Harvest dates			
	Hybrid alfalfa		Red clover	
	2016	2017	2016	2017
1	17.06	17.06	23.06	17.06
2	27.07	26.07	10.08	24.07
3	17.09	23.09	09.10	23.09

Values of Selianinov's hydrothermal coefficient (K) are presented in Table 5. It was assumed that extreme conditions took place when its value was below 0.7 and above 2.5 (Skowera and Puła, 2004).

Optimal temperature and moisture conditions were only in April 2015 and in September 2016. In the remaining months of the growing periods they were not as favourable, varying from extremely dry in August 2016 to extremely wet in May 2016. Throughout the experiment the best conditions were at the beginning of each growing season. It can be concluded that the most difficult situation for plants was in 2016, when, apart from May and the end of the growing season, the weather ranged from moderately dry to extremely dry. The growing season of 2017 was characterized by a lack of a period with optimal conditions for plant growth. A high level of precipitation in July (K = 2.15) and drought in most of the remaining months hindered proper growth and development of plants (Fig. 1).

2.4. Statistical analysis

The results of the multi-factor experiment with 24 experimental units were processed statistically using repeated measures analysis of variance; the same forage parameters were measured during each growing cycle. The Fisher-Snedecor test was done to determine the significance of the effect of experimental factors on the parameters. Tukey's test was used to compare differences between means at the $HSD_{0.05}$ significance level. All the calculations were made with the Statistica 13 – 2017 program. In tables homogenous groups are marked with letters. Means in lines and columns marked with the same letters are not significantly different.

3. Results

3.1. The yield of hybrid alfalfa and red clover

The 13.90 Mg ha^{-1} dry matter yield of hybrid alfalfa, average across all treatments, was significantly higher than the average yield of red clover with 9.94 Mg ha^{-1} (Table 6). In the second year, 2017, the average yield of hybrid alfalfa was 16.20 Mg ha^{-1}, while the yield of red clover stayed at a similar level (9.94 Mg ha^{-1}) as in 2016.

The plants responded to different doses of Tytanit with significant yield diversity. The largest 35.6% increase relative to control in the dry matter yield, average for both species, was on plots treated with the highest dose of the biostimulant (0.6 dm^3), but smaller doses (0.2 and 0.4 dm^3) also contributed to its significant increase (on average by 17.6%). It should be noted, however, that in the latter case the yield was about 18.0% lower than the weight of plants with the highest dose of Tytanit.

The yield of hybrid alfalfa and red clover was dependent on the harvest (Table 7). The highest dry matter yield across both species and all treatments was in the first harvest (4.71 Mg ha^{-1}). This value was 11.6% higher than the amount of dry matter in the second harvest and 58.1% higher than in the third. Red clover average yields were similar in all three harvests, ranging from 2.74 to 3.87 Mg ha^{-1}. In turn, hybrid alfalfa had a tendency to produce the most dry matter in the first harvest and the least in the third one.

3.2. Hybrid alfalfa and red clover nutritional value

3.2.1. Total protein content in dry matter

The data presented in Table 8 indicate that significantly greater total protein content as the average across treatments was in the dry matter of hybrid alfalfa (213 g kg^{-1}) than in red clover (188 g kg^{-1} DM). In addition, hybrid alfalfa total protein concentration was higher in the second year (217 g kg^{-1}), but in red clover it was similar in both growing periods (an average of 188.5 g kg^{-1}). The applied doses of the biostimulant diversified protein content in the dry matter of plants. Its highest amount (an average of 202 g kg^{-1}) was reported on the plots with 0.4 and 0.6 dm^3 doses. Significantly less protein was reported in plants treated with the smallest dose of Tytanit. The studies showed no interaction between the growing season and average protein content.

The highest average total protein content across treatments and species was in the first harvest (216 g kg^{-1}); it was lower in the second (201 g kg^{-1}) and in the third (184 g kg^{-1}), with a statistically significant difference between those values.

Table 5
The value of Sielianinov's hydrothermal coefficient (K) in the growing season.

Years	Months						
	April	May	June	July	August	September	October
2015	1.36 (o)	1.87 (mw)	1.64 (mw)	0.59 (sd)	1.92 (mw)	0.64 (sd)	0.12 (ed)
2016	1.22 (md)	2.63 (sw)	0.87 (d)	1.08 (md)	0.18 (ed)	1.46 (o)	1.94 (mw)
2017	1.89 (mw)	0.82 (d)	1.02 (md)	2.15 (w)	1.05 (md)	0.36 (ed)	7.65 (ew)

$K \le 0.4$ extreme drought (ed), $0.4 < K \le 0.7$ severe drought (sd), $0.7 < K \le 1.0$ drought (d), $1.0 < K \le 1.3$ moderate drought (md), $1.3 < K \le 1.6$ optimal (o), $1.6 < K \le 2.0$ moderately wet (mw), $2.0 < K \le 2.5$ wet (w), $2.5 < K \le 3.0$ severely wet (sw), $K > 3.0$ extremely wet (ew).
2015 - the year in which the experiment was established, 2016 and 2017 - years of full use of experience.

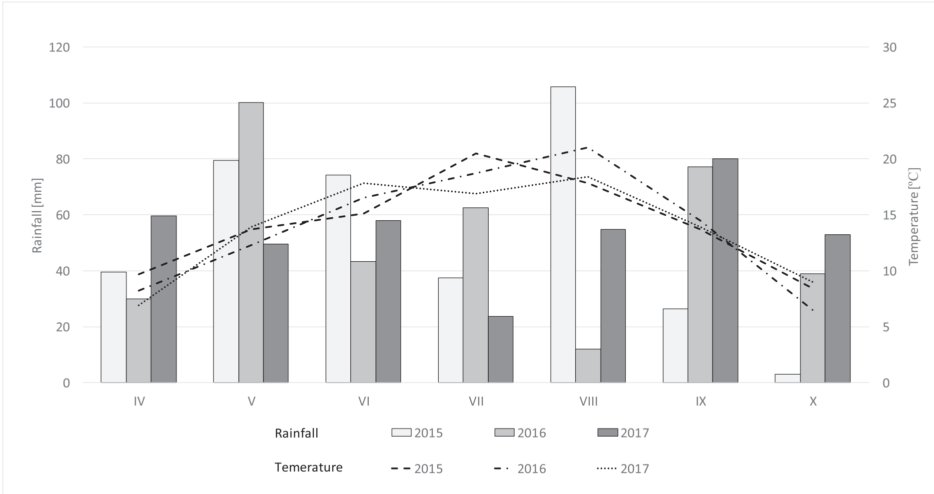

Fig. 1. Average of air temperature and sum of atmospheric precipitation in the months of the growing seasons during the research.

Table 6
The effect of Tytanit on the yield of hybrid alfalfa and red clover dry matter across growing seasons (Mg ha^{-1}).

Species	Treatments	Growing season		Means
		2016	2017	
Alfalfa	$Ti_{0.0}$	10.29 ± 1.75 Bb	13.89 ± 2.45 Ac	12.09 ± 2.13 bc
	$Ti_{0.2}$	9.33 ± 0.97 Bbc	17.46 ± 2.31 Aa	13.40 ± 1.85 b
	$Ti_{0.4}$	10.77 ± 1.96 Bb	15.90 ± 2.74 Ab	13.34 ± 2.01 b
	$Ti_{0.6}$	16.02 ± 2.04 Ba	17.55 ± 1.87 Aa	16.79 ± 2.98 a
Red Clover	$Ti_{0.0}$	8.31 ± 0.87 Bc	8.01 ± 1.23 Ad	8.16 ± 0.94 d
	$Ti_{0.2}$	11.88 ± 1.22 Ab	8.70 ± 0.82 Ad	10.29 ± 1.37 c
	$Ti_{0.4}$	12.48 ± 1.81 Ab	8.76 ± 1.65 Ad	10.62 ± 1.87 c
	$Ti_{0.6}$	11.40 ± 1.99 Ab	9.99 ± 1.68 Ad	10.70 ± 2.08 c
Treatment means				
$Ti_{0.0}$		9.30 ± 1.47 Bc	10.95 ± 0.91 Ab	10.13 ± 1.78 c
$Ti_{0.2}$		10.61 ± 1.74 Bb	13.08 ± 2.11 Aa	11.85 ± 2.11 b
$Ti_{0.4}$		11.63 ± 1.32 Bb	12.33 ± Aa	11.98 ± 2.14 b
$Ti_{0.6}$		13.71 ± 2.41 Aa	13.77 ± Aa	13.74 ± 2.75 a
Species means				
Alfalfa		11.60 ± 1.87 Ba	16.20 ± 2.87 Aa	13.90 ± 2.11 a
Clover		11.02 ± 1.87 Aa	8.87 ± 1.87 Ab	9.94 ± 2.07 b
Means		11.31 ± 2.13 B	12.54 ± 1.45 A	

Means in columns marked with the same small letters do not differ significantly. Means in rows marked with the same capital letters do not differ significantly. Standard deviation (±SD).

3.2.2. Crude fibre content in the dry matter of plants

As the average across all treatments and harvests hybrid alfalfa had higher crude fibre content (256 g kg^{-1}) in the dry matter than red clover (Table 9). This content was affected by biostimulant doses. The greatest amount of fibre was in control plants (250 g kg^{-1}) and in those treated with a dose of 0.2 dm^3 (247 g kg^{-1}). In turn, dry matter of plants from plots treated with Tytanit doses of 0.4 and 0.6 dm^3 had a significantly lower (on average by 5.1%) concentration of that component. However, for both species the content of crude fibre was substantially diversified as a result of an interaction between treatments and harvests. In the first and second harvests the 0.6 dm^3 dose of Tytanit caused a significant decline in fibre content, by 6.8% and 8.9% relative to control. In the third harvest the same dose also caused a decrease in the content of this component (by 3.7%), but the difference was not statistically significant.

The experiment confirmed the view that harvest time affects crude fibre content in dry matter. As the average across all treat-

Table 7
The effect of Tytanit on the yield of hybrid alfalfa and red clover dry matter yield across harvests (Mg ha^{-1}).

Species	Treatment	Harvest		
		1	2	3
Alfalfa	$Ti_{0.0}$	5.07 ± 0.78 Aab	4.62 ± 0.34 Aa	2.39 ± 0.21 Bb
	$Ti_{0.2}$	6.53 ± 0.98 Aa	4.05 ± 0.74 Ba	2.82 ± 0.23 Cb
	$Ti_{0.4}$	6.06 ± 1.01 Aa	4.56 ± 0.32 Ba	2.72 ± 0.31 Cb
	$Ti_{0.6}$	6.81 ± 0.59 Aa	5.05 ± 0.45 Ba	4.93 ± 0.52 Ba
Clover	$Ti_{0.0}$	3.22 ± 0.67 Ab	2.59 ± 0.37 Ab	2.35 ± 0.24 Ab
	$Ti_{0.2}$	3.64 ± 0.49 Bb	4.14 ± 0.43 Aa	2.50 ± 0.61 Cb
	$Ti_{0.4}$	3.25 ± 0.72 Bb	4.75 ± 0.49 Aa	2.62 ± 0.21 Bb
	$Ti_{0.6}$	3.20 ± Ab	4.00 ± 0.39 Aa	3.50 ± 0.41 Aab
Treatment means				
$Ti_{0.0}$		4.15 ± 0.64 Ac	3.60 ± 0.29 Bc	2.37 ± 0.28 Bc
$Ti_{0.2}$		5.08 ± 0.59 Aa	4.10 ± 0.67 Bb	2.66 ± 0.34 Cb
$Ti_{0.4}$		4.66 ± Ab	4.66 ± 0.65 Aa	2.67 ± 0.50 Bb
$Ti_{0.6}$		5.01 ± 0.72 Aa	4.53 ± 0.71 Ba	4.22 ± 0.38 Ba
Species means				
Alfalfa		6.12 ± 1.18 Aa	4.57 ± 0.61 Ba	3.21 ± 0.69 Ca
Clover		3.33 ± 0.85 Ab	3.87 ± 0.76 Ab	2.74 ± 0.36 Ab
Means		4.71 ± 1.02 A	4.22 ± 0.72 B	2.98 ± 0.64 C

Means in columns marked with the same small letters do not differ significantly. Means in rows marked with the same capital letters do not differ significantly. Standard deviation (±SD).

Table 8
The effect of Tytanit on total protein content in hybrid alfalfa and red clover dry matter across growing seasons and harvests (g kg^{-1}).

Treatment	Species		Growing season		Harvest			Means
	Alfalfa	Clover	2016	2017	1	2	3	
$Ti_{0.0}$	214 ± 6.41 Aa	184 ± 7.07 Bb	194 ± 6.17 Ab	203 ± 6.47 Aa	216 ± 7.08 Aab	201 ± 6.11 Aab	179 ± 4.74 Bc	199 ± 6.41 b
$Ti_{0.2}$	210 ± 4.89 Ab	188 ± 3.59 Bab	198 ± 4.43 Ab	200 ± 6.54 Aa	211 ± 4.47 Ab	194 ± 4.88 Ba	193 ± 3.98 Ba	199 ± 7.32 b
$Ti_{0.4}$	217 ± 7.02 Aa	188 ± 3.12 Bab	208 ± 3.57 Aa	198 ± 2.45 Aa	227 ± 6.87 Aa	200 ± 5.65 Ba	182 ± 5.01 Cb	202 ± 5.32 a
$Ti_{0.6}$	212 ± 3.98 Aab	192 ± 3.01 Aa	200 ± 4.95 Aa	203 ± 3.69 Aa	213 ± 8.09 Ab	209 ± 4.56 Aa	185 ± 6.11 Bab	202 ± 4.12 a
Means	213 ± 2.58 A	188 ± 2.82 B	200 ± 5.09 A	201 ± 2.12 A	216 ± 6.17 A	201 ± 5.33 B	184 ± 5.21 C	

Means in columns marked with the same small letters do not differ significantly. Means in rows marked with the same capital letters do not differ significantly. Standard deviation (±SD)

ments its lowest content was in plants from the spring harvest (the first harvest) and autumn harvest (the third), with an average of 236 g kg^{-1}.

3.2.3. Digestibility of dry matter

Red clover dry matter digestibility (Table 10) with 57.7%, as the average across treatments and growing seasons, was greater than that of hybrid alfalfa (55.5%). Different doses of the growth regulator did not diversify this parameter much. As the average across all treatments it ranged from 55.9 to 57.6%. In turn, the statistical analysis showed significant variation of the characteristics in particular harvests. The least digestible was the dry matter of plants collected in the second harvest (53.2%). The plant material from the first and third harvests had significantly higher digestibility, with 58% in the first harvest and 58.6% in the third one.

Low dry matter digestibility of hybrid alfalfa and red clover in the present experiment resulted from the fact that there were only three harvests in the growing season.

3.2.4. Concentration of selected macronutrients in plant dry matter

Calcium content, as the average effect of treatments, was similar in both plant species (Table 11), and with 20.5 g kg^{-1} in hybrid alfalfa and 19. 6 g kg^{-1} in red clover it did not differ significantly. Additionally, no significant differences between calcium content in plant dry matter were observed across growing seasons. However, this content was diversified in a statistically significant way in response to different Tytanit doses. The highest calcium content

Table 9
The effect of Tytanit on crude fibre content in hybrid alfalfa and red clover dry matter across growing seasons and harvests (g kg^{-1}).

Treatment	Species		Growing season		Harvest			Means
	Alfalfa	Clover	2016	2017	1	2	3	
$Ti_{0.0}$	258 ± 7.12 Aa	244 ± 4.78 Aab	254 ± 8.11 Aa	247 ± 7.81 Ba	237 ± 5.47 Ba	269 ± 6.71 Aa	246 ± 7.08 Ba	250 ± 5.23 a
$Ti_{0.2}$	264 ± 8.31 Aa	231 ± 5.69 Bb	248 ± 6.78 Aa	247 ± 3.46 Aa	242 ± 6.45 ABa	263 ± 5.55 Aab	239 ± 6.51 Bab	247 ± 6.74 a
$Ti_{0.4}$	247 ± 4.56 Ab	228 ± 7.01 Bbc	241 ± 9.01 Ab	234 ± 8.21 Ab	237 ± 7.45 ABa	252 ± 6.71 Abc	225 ± 8.14 Bb	238 ± 7.14 b
$Ti_{0.6}$	254 ± 6.12 Aa	218 ± 3.98 Bc	234 ± 4.55 Ab	236 ± 4.63 Ab	222 ± 6.12 Bb	247 ± 8.21 Ac	237 ± 7.28 Aab	235 ± 4.98 b
Means	256 ± 6.17 A	230 ± 9.28 b	244 ± 7.49 A	241 ± 6.04 A	234 ± 7.50 B	258 ± 8.96 A	237 ± 8.21 B	

Means in columns marked with the same small letters do not differ significantly. Means in rows marked with the same capital letters do not differ significantly. Standard deviation (±SD).

Table 10
The effect of Tytanit on dry matter digestibility of hybrid alfalfa and red clover across growing seasons and harvests (%).

Treatment	Species		Growing season		Harvest			Means
	Alfalfa	Clover	2016	2017	1	2	3	
$Ti_{0.0}$	55.9 ± 0.84 Ba	59.3 ± 0.54 Aa	57.0 ± 0.60 Aa	58.2 ± 0.81 Aa	57.0 ± 0.62 ABa	56.2 ± 0.81 Ba	59.6 ± 0.63 Aa	57.6 ± 1.05 a
$Ti_{0.2}$	55.8 ± 0.27 Ba	57.3 ± 0.91 Aa	56.6 ± 0.29 Aa	56.5 ± 0.74 Aa	59.7 ± 0.74 Aa	53.0 ± 0.67 Bb	56.9 ± 0.74 ABa	56.5 ± 0.98 a
$Ti_{0.4}$	55.2 ± 0.35 Ba	56.6 ± 0.67 Aa	54.9 ± 0.71 Aa	56.9 ± 0.64 Aa	57.1 ± 0.36 Aa	52.2 ± 0.53 Bb	58.4 ± 0.54 Aa	55.9 ± 0.90 a
$Ti_{0.6}$	55.3 ± 0.41 Ba	57.6 ± 0.47 Aa	55.4 ± 0.50 Aa	57.5 ± 0.47 Ba	58.2 ± 0.74 Aa	51.5 ± 0.75 Bb	59.5 ± 0.38 Aa	56.4 ± 1.11 a
Means	55.5 ± 0.19 B	57.7 ± 0.99 A	56.0 ± 0.85 B	57.2 ± 1.18 A	58.0 ± 1.08 A	53.2 ± 0.84 B	58.6 ± 0.82 A	

Means in columns marked with the same small letters do not differ significantly. Means in rows marked with the same capital letters do not differ significantly. Standard deviation (±SD).

(22.5 g kg^{-1}), average for both species, was in plants treated with a Tytanit dose of 0.4 dm^3 per ha. This value was 30.1% higher than that in control plots. Plants with the smallest dose had lower calcium content in the biomass (20. 7 g kg^{-1}). In turn, the largest dose of the biostimulant decreased it to 19.8 g kg^{-1}, which was lower than in plants sprayed with a dose of 0.4 dm^3. It must be stressed, however, that each Tytanit treatment resulted in a significant increase relative to control in calcium content.

Calcium content in plant dry matter did not substantially vary from harvest to harvest. The average calcium content ranged from 19.2 in the third one to 20. 8 g kg^{-1} in the second. In turn, the analysis of the interaction between treatments and harvests showed that a dose of 0.4 dm^3 in the second and third one caused the largest increase in the concentration of this macronutrient in plant dry matter in relation to control.

Magnesium content as the average across treatments was at the same level of 3.30 g kg^{-1} in the dry matter of both hybrid alfalfa and red clover (Table 11). In addition, a significantly higher amount of magnesium, average for species and treatments, was in the second year of the experiment (3.40 g kg^{-1}). The statistical analysis also showed that plants treated with 0.2 and 0.4 dm^3 Tytanit doses responded with significantly higher magnesium content, 40.7% more than in control plants. However, it was not significantly affected by the largest dose. Furthermore, no significant interaction between the harvest and the magnesium content in the plants was observed.

The highest amounts of phosphorus were in plants to which the 0.4 dm^3 (3.37 g kg^{-1}) and 0.2 dm^3 (3.34 g kg^{-1}) doses were applied. These values did not differ from each other significantly, but they were higher than phosphorus content in control (3.05 g kg^{-1}) and higher than the 3.09 g kg^{-1} value in plants with the highest dose of Tytanit.

There was an interaction between phosphorus content (Table 11) and harvest time. The largest phosphorus content was in the plant material of the spring harvest (3.44 g kg^{-1}). In the second and third harvest its average value was 3.10 g kg^{-1}. In addition, for both species substantially higher values of phosphorus content were in the first harvest of plants treated with 0.4 (3.77 g kg^{-1}) and 0.2 dm^3 (3.73 g kg^{-1}) doses. Significantly higher phosphorus content, as the average across all treatments, was in hybrid alfalfa (3.30 g kg^{-1}). In the dry matter of red clover the average concentration of this element was 3. 12 g kg^{-1}. In addition, the highest amount of phosphorus in hybrid alfalfa was in the second year of the experiment (3.54 g kg^{-1}).

Potassium content in the dry matter of hybrid alfalfa as the average across treatments and years of research amounted to 17.2 g kg^{-1}, and in red clover it was 17.6 g kg^{-1} (Table 11). These values did not differ significantly, but the highest content of potassium in the dry matter as the average across treatments and species was in the first year (18.0 g kg^{-1}). Tytanit doses diversified the amounts of this macronutrients. In plants with Tytanit doses of 0.2 and 0.4 dm^3 its content was the largest (19.7 and 19. 6 g kg^{-1}, respectively). Its smaller amounts (17.1 g kg^{-1}) were recorded in the dry matter of plants treated with a dose of 0.6 dm^3. In turn, the smallest content was in control plants (13.4 g kg^{-1}). It was the biggest in the first harvest (18.0 g kg^{-1}), but in the next harvest the average concentration was lower, with 17.2 g kg^{-1}.

3.2.5. The ratios of selected microelements in plant dry matter

The ratios of K: (Ca + Mg) in the dry matter of hybrid alfalfa and red clover with 0.73 and 0.77, respectively, did not differ significantly (Table 12). In the first year the ratio of potassium ions to the sum of calcium and magnesium ions, as the average across

Table 11
The effect of Tytanit on calcium, magnesium, phosphorus, and potassium content in hybrid alfalfa and red clover dry matter (g kg^{-1}).

Treatments	Species		Year		Harvest			Means
	Alfalfa	Clover	2016	2017	1	2	3	
Ca								
$Ti_{0.0}$	17.1 ± 1.12 Ac	17.5 ± 1.07 Ac	17.2 ± 0.78 Ab	17.4 ± 0.68 Ac	17.6 ± 1.08 Ab	17.3 ± 1.21 Ac	16.9 ± 0.47 Ac	17.3 ± 0.28 d
$Ti_{0.2}$	22.1 ± 0.87 Aa	19.3 ± 1.14 Bb	21.4 ± 1.47 Aa	19.9 ± 0.67 Ab	21.9 ± 0.94 Aa	20.9 ± 1.24 Ab	19.2 ± 0.60 Bb	20.7 ± 0.72b
$Ti_{0.4}$	22.8 ± 1.10 Aa	22.3 ± 0.72 Aa	22.7 ± 1.65 Aa	22.4 ± 0.56 Aa	21.3 ± 1.22 Ba	24.3 ± 0.89 Aa	22.0 ± 0.52 Aa	22.5 ± 0.49 a
$Ti_{0.6}$	20.3 ± 0.86 Ab	19.2 ± 1.74 Ab	18.3 ± 1.12 Ab	21.2 ± 0.71 Aa	19.9 ± 1.04 ABa	20.6 ± 1.20 Ab	18.8 ± 0.63 Bb	19.8 ± 0.98 c
Means	20.5 ± 2.20 A	19.6 ± 2.98 A	19.9 ± 2.23 A	20.2 ± 1.84 A	20.2 ± 1.65 A	20.8 ± 1.45 A	19.2 ± 1.82 A	
Mg								
$Ti_{0.0}$	2.80 ± 0.46 Ab	2.60 ± 0.11 Ab	2.60 ± 0.30 Ab	2.80 ± 0.90 Ab	2.80 ± 1.01 Ab	2.70 ± 0.61 Ab	2.60 ± 0.29 Ab	2.70 ± 0.11 b
$Ti_{0.2}$	3.90 ± 0.52 Aa	3.80 ± 0.34 Aa	3.70 ± 0.32 Aa	3.90 ± 0.71 Aa	3.90 ± 0.67 Aa	3.80 ± 0.41 Aa	3.80 ± 0.31 Aa	3.80 ± 0.09 a
$Ti_{0.4}$	3.80 ± 0.28 Aa	3.90 ± 0.71 Aa	3.80 ± 0.41 Aa	3.80 ± 0.63 Aa	3.80 ± 0.71 Aa	3.80 ± 0.65 Aa	3.80 ± 0.61 Aa	3.80 ± 0.13 a
$Ti_{0.6}$	2.80 ± 0.35 Ab	3.10 ± 0.35 Aab	2.80 ± 0.19 Ab	3.10 ± 0.47 Ab	2.70 ± 0.46 Ab	3.20 ± 0.50 Ab	3.00 ± 0.50 Ab	3.00 ± 0.21 b
Means	3.30 ± 0.52 A	3.30 ± 0,49 A	3.30 ± 0.74 B	3.40 ± 0.41 A	3.30 ± 0.36 A	3.40 ± 0.60 A	3.30 ± 0.42 A	
P								
$Ti_{0.0}$	3.11 ± 0.21 Ab	3.01 ± 0.23 Bb	3.13 ± 0.11 Aa	2.98 ± 0.19 Ab	3.16 ± 0.27 Ab	3.08 ± 0.41 Aa	2.93 ± 0.39 Ab	3.05 ± 0.46 b
$Ti_{0.2}$	3.50 ± 0.47 Aa	3.18 ± 0.31 Bab	2.97 ± 0.31 Ba	3.72 ± 0.37 Aa	3.73 ± 0.29 Aa	3.11 ± 0.17 Ba	3.19 ± 0.78 Bab	3.34 ± 0.28 a
$Ti_{0.4}$	3.48 ± 0.17 Aa	3.25 ± 0.41 Ba	3.11 ± 0.20 Ba	3.63 ± 0.29 Aa	3.77 ± 0.30 Aa	3.05 ± 0.24 Ba	3.29 ± 0.27 Ba	3.37 ± 0.18 a
$Ti_{0.6}$	3.12 ± 0.11 Ab	3.05 ± 0.27 Ab	3.08 ± 0.30 Aa	3.09 ± 0.31 Ab	3.10 ± 0.28 Ab	3.17 ± 0.16 Aa	2.98 ± 0.19 Ab	3.09 ± 0.32 b
Means	3.30 ± 0.19 A	3.12 ± 0.20 B	3.07 ± 0.89 B	3.36 ± 0.47 A	3.44 ± 0.23 A	3.10 ± 0.18 B	3.10 ± 0.20 B	
K								
$Ti_{0.0}$	13.7 ± 1.12 Ac	13.0 ± 0.78 Ac	13.5 ± 1.13 Ac	13.2 ± 1.71 Ac	13.8 ± 1.79 Ac	12.9 ± 1.45 Ac	13.4 ± 1.17 c	13.4 ± 0.71 b
$Ti_{0.2}$	20.0 ± 0.98 Aa	19.5 ± 1.87 Aab	20.1 ± 1.31 Aa	19.3 ± 0.91 Aa	20.6 ± 1.36 Aa	19.5 ± 1.49 Aa	19.0 ± 1.24 Aa	19.7 ± 0.87 a
$Ti_{0.4}$	19.1 ± 1.04 Aa	20.0 ± 2.01 Aa	20.7 ± 1.41 Aa	18.5 ± 1.08 Ba	19.7 ± 0.93 Aa	19.3 ± 1.75 Aa	19.7 ± 1.32 Aa	19.6 ± 0.87 a
$Ti_{0.6}$	16.4 ± 0.98 Ab	17.8 ± 0.91 Ab	17.8 ± 0.71 Ab	16.4 ± 1.24 Ab	17.7 ± 1.19 Aa	16.6 ± 1.37 Ab	17.0 ± 1.09 Ab	17.1 ± 0.67 b
Means	17.2 ± 2.46 A	17.6 ± 2.76 A	18.0 ± 2.41 A	16.8 ± 2.09 B	18.0 ± 2.11 A	17.1 ± 1.89 B	17.3 ± 2.07 B	

Means in columns marked with the same small letters do not differ significantly. Means in rows marked with the same capital letters do not differ significantly. Standard deviation (±SD).

treatment and species, was significantly higher than in the next year. The statistical analysis also showed that the ratio was diversified in response to Tytanit doses. The lowest dose of Tytanit resulted in a significant widening of the K: (Ca + Mg) ratio, from 0.67 in control to 0.81 for plants treated with a 0.2 dm^3 dose. The largest K: (Ca + Mg) ratio was recorded in the dry matter of plants collected in the first (0.76) and third (0.77) harvests. For the second one the 0.71 value was significantly the lowest. In addition, as is apparent from the analysis of the interaction between treatments and harvest, Tytanit did not affect the differences between harvests.

The average ratio of Ca: P in the dry matter of alfalfa and red clover was very high and amounted to 6.26 (Table 12). The ratio increased in consecutive growing periods from 6.48 in 2016 to 6.04 in 2017. Significant differences were also caused by Tytanit doses. The highest average ratio (6.74) was obtained in the dry matter of plants treated with a dose of 0.4 dm^3. It is worth noting that this dose also contributed to a substantial increase in the content of calcium and phosphorus (Table 11); accumulation of calcium in the dry matter of plants increased by 30.1% relative to control and phosphorus by only about 10.5%, which led directly to a very large value of the Ca: P ratio in plants treated with Tytanit. The smallest and the largest doses of the biostimulant also caused a significant widening of the ratio relative to control. When plants were harvested three times a year the highest ratio of calcium to phosphorus (6.70) was in the dry matter of the second harvest. In turn, the smallest possible ratio of these ions (5.88) was in the first harvest. An analysis of the interaction between treatments and harvests indicated that a stable value (with not significant differences) of the Ca: P ratio in harvests was only in control plants, and Tytanit foliar application did not stabilize the ratio in individual harvests.

4. Discussion

4.1. The yield of hybrid alfalfa and red clover

Godlewska and Ciepiela (2018) examined the impact of biostimulants and varied doses of mineral nitrogen on the hybrid alfalfa yield and obtained similar results to those recorded in the present experiment. The authors found that a Tytanit dose of 0.4 dm^3 ha^{-1} caused a 15.6% increase relative to control in the

Table 12
The effect of Tytanit on K:(Ca + Mg) and Ca:P ratios in hybrid alfalfa and red clover dry matter.

Treatments	Species		Growing season		Harvest			Means
	Alfalfa	Clover	2016	2017	1	2	3	
K:(Ca + Mg)								
$Ti_{0.0}$	0.70 ± 0.06 Aab	0.65 ± 0.03 Bb	0.68 ± 0.03 Ab	0.65 ± 0.03 Ab	0.68 ± 0.04 Ab	0.65 ± 0.07 Ab	0.69 ± 0.03 Ac	0.67 ± 0.02 b
$Ti_{0.2}$	0.78 ± 0.03 Ba	0.85 ± 0.06 Aa	0.80 ± 0.04 Aa	0.81 ± 0.04 Aa	0.80 ± 0.05 Aa	0.79 ± 0.03 Ba	0.83 ± 0.07 Aa	0.81 ± 0.03 a
$Ti_{0.4}$	0.73 ± 0.02 Aab	0.77 ± 0.05 Aab	0.78 ± 0.06 Aa	0.71 ± 0.06 Bab	0.78 ± 0.06 Aa	0.69 ± 0.03 Bb	0.76 ± 0.06 Ab	0.75 ± 0.04 ab
$Ti_{0.6}$	0.71 ± 0.05 Bab	0.81 ± 0.06 Aa	0.84 ± 0.04 Aa	0.68 ± 0.07 Bb	0.78 ± 0.04 Ab	0.70 ± 0.08 Bb	0.78 ± 0.03 Ab	0.76 ± 0.03 ab
Means	0.73 ± 0.07 A	0.77 ± 0.09 A	0.78 ± 0.06 A	0.71 ± 0.05 B	0.76 ± 0.05 A	0.71 ± 0.06 B	0.77 ± 0.04 A	
Ca:P								
$Ti_{0.0}$	5.46 ± 0.42 Bb	5.83 ± 0.42 Ab	5.50 ± 0.36 Ab	5.84 ± 0.32 Ab	5.57 ± 0.52 Ab	5.62 ± 0.22 Ab	5.77 ± 0.34 Ab	5.67 ± 0.12 b
$Ti_{0.2}$	6.52 ± 0.29 Aa	6.09 ± 0.28 Bab	7.20 ± 0.50 Aa	5.35 ± 0.31 Bb	5.87 ± 0.49 Aab	6.72 ± 0.39 Ba	6.02 ± 0.13 Aab	6.28 ± 0.24 a
$Ti_{0.4}$	6.66 ± 0.27 Aa	6.85 ± 0.31 Aa	7.30 ± 0.51 Aa	6.17 ± 0.30 Bab	5.65 ± 0.20 Ab	7.97 ± 0.65 Ba	6.69 ± 0.20 Aa	6.74 ± 0.81 a
$Ti_{0.6}$	6.50 ± 0.41 Aa	6.29 ± 0.51 Aa	5.94 ± 0.60 Bb	6.86 ± 0.47 Ab	6.41 ± 0.31 Aa	6.50 ± 0.73 Bab	6.31 ± 0.27 Aa	6.40 ± 0.21 a
Means	6.25 ± 0.35 A	6.27 ± 0.37 A	6.48 ± 0.78 A	6.04 ± 0.30 B	5.88 ± 0.38 B	6.70 ± 0.71 A	6.22 ± 0.11 AB	

Means in columns marked with the same small letters do not differ significantly. Means in rows marked with the same capital letters do not differ significantly. Standard deviation (±SD).

yield, as an average for growing seasons and mineral fertilizer treatments. In addition, mineral fertilizer increased the effect of Tytanit. The influence of the biostimulant on yield growth of fodder plants was also noted by other authors. Radkowski and Radkowska (2010) in their studies based on the application of different doses of Tytanit to permanent meadows reported a significant increase in the plant dry matter yield. They found that the biggest effect was after treating plants with 0.04 and 0.08% solutions of Tytanit, with an yield increase of 52% and 40%, respectively, while the use of a 0.02% solution increased the yield by 26%.

Studies done by Radkowski et al., (2015) demonstrated that an increase in the *Phleum pratense* L. yield after Tytanit application was due to its influence on plant morphological parameters. The authors found that the highest dose (0.8 dm^3 ha^{-1}) contributed to an increase in the length of stems, florescence, and leaf blades of this species, leading to 19.5% yield growth. In their experiment Prusiński and Kaszkowiak (2005) observed a 7.4–18% increase of the lupine seed and straw yield after applying Tytanit twice at a concentration of 0.02–0.04%.

Different results were obtained by Jaberzadeh et al. (2013) and Whitted-Haag et al. (2014). Jaberzadeh et al. (2013) observed that higher Ti concentrations (0.03%) in the spray liquid applied to *Triticum aestivum* contributed to a reduction of the plant height. According to Whitted-Haag et al. (2014) titanium application (0, 25, 50, 75, and 100 mg dm^{-3}) to annual bedding plants (pelargonium, *Impatiens walleriana*, pansy, and petunia) only slightly affected their height but significantly increased the stem diameter. It was found in the research that the stem diameter of *Impatiens walleriana* showed a linear decrease when the concentration of titanium in the spray liquid increased, from 8.9 mm in plants on control units to 7.4 mm in plants treated with Tytanit at a concentration of 100 mg dm^{-3}. In addition, according to the same authors, the number of leaves per plant was significantly reduced, from 31 in control to 27 leaves in plants treated with Ti.

Raskar and Lawares (2013) and Clement et al. (2013) found that a low dose of Tytanit stimulated seed germination and early growth of seedlings, while higher doses caused an opposite effect. The available literature (Cigler et al., 2010; Giménez et al., 1990; Hrubý et al., 2002; Radkowski, 2013; Wadas and Kalinowski, 2017b; Yaghoubi et al., 2000) suggests that titanium application has a beneficial effect on the processes of photosynthesis, with an increase in the concentration of chlorophyll in leaf blades, better nitrogen uptake by cells, and growth of the SPAD index exceeding 20%. In addition, one of the consequences of titanium application to plants is an increase in glucose production, which translates into faster synthesis of biopolymers such as cellulose and starch. This can be treated as a secondary effect of titanium application to plants. Positive effects of its use on the activation of plant physiological process leading to an increase in their yield were also observed by Dobromilska (2007), Kleiber and Markiewicz (2013), Kužel et al. (2003), and Tlustoš et al. (2005).

4.2. Nutritional value

4.2.1. Protein content in plant dry matter

Although no significant changes in protein content throughout the present experiment were recorded, different results were presented by Gaweł and Żurek (2003). Studying protein content of three alfalfa varieties they demonstrated a decrease in its amount in consecutive years. In turn, Godlewska and Ciepiela (2018) recorded an upward trend in protein content in the dry matter of hybrid alfalfa in consecutive growing seasons. At the same time the authors found that the application a Tytanit dose of 0.4 dm^3 kg^{-1} resulted in a 6.6% increase in protein content in the dry matter of plants. Low concentration of total protein stemmed from a late harvest, which was a consequence of harvesting alfalfa three times a year. Studies of Andrzejewska et al. (2013) showed that delaying an alfalfa harvest from the beginning of the budding stage to the flowering stage resulted in a decrease of total protein content in the dry matter by 20 g kg^{-1} on average In addition, the authors found that a decrease in the concentration of protein caused an increase in the content of fibre, with NDF concentration increasing by 14.8% and ADF by 17.4%. Total protein content in the present experiment was comparable with the results provided by Wilczek and Ćwintal (1996). In the studies of Radkowski and Grygierzec (2006) examining selected varieties of hybrid alfalfa and *Medicago sativa* total protein content ranged from 170 to 220 g kg^{-1} DM. In turn, Skrzyniarz and Ufnowska (1993) studied total protein content in *Fabaceae* plants and found it to be 190 g kg^{-1} DM on average.

4.2.2. Digestibility of dry matter

According to Stachowicz (2010) plant material with proper nutrient parameters should have its digestibility at around 65%, which means, according to Pawlak (1990), that 65% of dry matter compounds should be absorbed from the digestive system. In the present experiment dry matter digestibility was higher in the second growing period, which was confirmed by the research of Gaweł (2012) on the digestibility of alfalfa mixtures with grasses. According to the literature (Gaweł and Żurek, 2003) the number of harvests during the growing period has a big effect on the digestibility of plant dry matter, which is closely related to the development stage of harvested plants and their pace of aging. Ścibior and Gaweł (2004) reported that dry matter digestibility of red clover harvested four times was 72%, and growing this species as a mixture of grasses raised digestibility up to 75%.

4.2.3. Concentration of selected macronutrients in plant dry matter

Nutrient content in the dry matter of plant mixtures depends, among other things, on the properties of individual species, their share in the mixture, the level of nitrogen application, the number of harvests and the time of the first harvest (Ćwintal, 2000; Gaweł and Żurek, 2003; Mastalerczuk, 2006; Mosimann et al., 1995; Nowak and Sowiński, 2007). In the present experiment Tytanit significantly increased calcium content in plants, and similar results were reported by Radkowski and Radkowska (2010). They observed that varied doses of Tytanit increased calcium content in the dry matter of meadow hay. The highest amounts of this chemical element were found in plants treated with 0.04% Tytanit solution in the spray liquid. In the first year of the experiment calcium content increased by 79%, with 133% in the second and 63% in the third, but this trend was not confirmed in the present experiment. Some authors (Pais, 1983; Skupień and Oszmiański, 2007; Wójcik, 2002) suggest that an increase in the content of certain minerals in the aboveground parts of plants as a response to a biostimulant containing titanium is caused by stronger development of the root system, especially by the extended hair zone. Consequently, this leads to an increased uptake of nutrients from the soil. In the literature there have also been reports (Kleiber and Markiewicz, 2013) that Tytanit applied in different doses does not affect calcium concentrations in the dry matter of some plant species, or, when applied in a very low amount it causes its decline.

The effect of Tytanit on an increase in magnesium content in hay meadow was noted by Radkowski and Radkowska (2010). The authors demonstrated that the application of a 0.04% solution of the biostimulant increased magnesium content in the plants by an average of 67%, while concentrations of 0.02 and 0.08% contributed to a substantial 32% increase of its accumulation. In tomato leaves the largest magnesium content was recorded when plants were treated with 960 g Ti ha^{-1}, but a small dose of 80 g Ti ha^{-1} reduced its amount relative to control (Kleiber and Markiewicz, 2013). In turn, Kalembasa et al. (2014) found the highest content of magnesium in leaf blades and petioles of celery by treating plants with 1.0, 1.2, and 2.4% Tytanit solutions, but a 3.6% solution and very low ones (0.001; 0.01; 0.1 and 1%) did not differentiate magnesium content.

According to Gaweł (2009) phosphorus content of 3 g kg^{-1} is considered optimal for animal feed. Effect of foliar application of biostimulants containing titanium on an increase in the content of phosphorus in vegetables and fruits was confirmed by Kleiber and Markiewicz (2013) and Wójcik and Wójcik (2001). In the present experiment it was found that there was an interaction between phosphorus content and the harvest.

Generally, forage plants provide excessive amounts of potassium (Falkowski et al., 2000; Gaweł, 2009). According to Gaweł and Madej (2008) a lack of precipitation can result in a large uptake of that chemical element by plants from the soil and in its excessive content in aboveground parts. The values recorded in the present experiment did not differ significantly, and according to literature (Ćwintal and Kościelecka, 2005a, b; Ćwintal and Wilczek, 2002) they are typical for the species used in the present experiment. Radkowski and Radkowska (2010) also recorded increased potassium content in plants treated with Tytanit. The authors found that the amount of potassium in the dry matter clearly increased (in some years up to 100%) on units where the concentrations of 0.02 and 0.04% were applied. Like in the present experiment, the highest concentration of titanium resulted in a slight but significant increase in the content of potassium. Different results were presented by Kalembasa et al. (2014), who reported an increase in the accumulation of potassium in relation to control in leaf celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers.). However, the authors did not record significant differences in its content as a response to different Tytanit doses. Similarly, Kleiber and Markiewicz (2013) did not note any significant effect of Tytanit doses on potassium content in tomato fruits.

4.2.4. The ratio of selected microelements in plant dry matter

According to Kalembasa et al. (2014) nutritional value of plants is determined not only by the content of individual macronutrients and micronutrients but also by their ratios. The same authors point out that in the case of macronutrients it is difficult to ensure the right ratios of individual chemical elements or their groups in plants. Research on small seed *Fabaceae* plants presented in numerous studies (Gaweł, 2005; Kallenbach et al., 2002; Kochanowska-Bukowska, 2003; Mastalerczuk, 2006; Mosimann et al., 1995; Nowak and Sowiński, 2007; Wilczek and Ćwintal, 2002) indicates that if they are intensively used they enrich their dry matter in nutrients, with changes in the value of the ratio of certain minerals. Many authors (Falkowski et al., 2000; Mastalerczuk, 2006; Nowak and Sowiński, 2007) claim that in assessing the value of food stuffs the optimal ratio of macronutrients in relation to each other is important, as deficiency or excess of some of them, in accordance with the antagonism and synergism of ions, may reduce feed efficiency and lead to metabolic disorders in animals. In addition, the authors point out that the ratio between potassium ions and divalent cations, i.e. magnesium and calcium, is very important for maintaining proper health of the consumers. According to Czapla and Nowak (1995) this ratio should not be higher than 1.62. Too high a value indicates a very big deficiency of calcium and magnesium in plant material. In turn, Ćwintal and Wilczek (2002) consider the limit of this ratio to be 2.2. The values of the K:(Ca + Mg) ratios recorded in the present experiment with 0.73 for hybrid alfalfa and 0.77 for red clover are within the limits provided by Gaweł (2009). According to the author for roughage the optimal value should vary between 0.66 and 0.98. In addition, as the author points out, the value of this feature is affected by the frequency of mowing and the time elapsed since planting. In the same research in the second year there was a decline in the ratio of K: (Ca + Mg), and in the present experiment there was a similar tendency. The influence of Tytanit on the ratio of macronutrients was also studied by Kalembasa et al. (2014). According to the authors Tytanit more than doubled the macronutrient ratio in leaf blades of leaf celery, which was the smallest on control units. The largest was on units with the largest dose of Tytanit applied together with NPK fertilizer, being twice bigger than in control plants. In addition, the above authors recorded a significant impact of the number of Tytanit foliar applications on this ratio. Double application of Tytanit expanded the ratio on average by 12.5% compared to the plants treated once.

The available literature shows (Gaweł, 2005; Kallenbach et al., 2002; Mastalerczuk, 2006; Mosimann et al., 1995; Nowak and Sowiński, 2007; Wilczek and Ćwintal, 2002; Szparaga et al., 2018) that the frequency and time of Fabaceae plant harvests affect their enrichment in nutrients and change the ratios of certain macronutrients. According to Ćwintal and Wilczek (2002) reduction in the number of Fabaceae harvests stabilizes the value of macronutrient ratio in the dry matter of plants.

Ćwintal and Wilczek (2002) and Falkowski et al. (2000) consider the ratio of calcium to phosphorus ions to be an important qualitative parameter of plant nutritional value. According to Falkowski et al. (2000) the optimal ratio in the dry matter of plants should be 2. In the present experiment the average ratio of Ca: P in the dry matter of alfalfa and red clover was 6.26, and according to Ćwintal and Wilczek (2002) and Gaweł (2009) such a high ratio is typical of small-seed Fabaceae plants, with their natural physiological tendency to accumulate large amounts of calcium in their aboveground parts. Therefore, a large ratio can indicate that phosphorus content is low, while there are excessive amounts of calcium in plant tissues. The above authors recorded a widening ratio of those cations in consecutive growing periods, and this trend was confirmed in the present experiment. Different results were provided by Wadas and Kalinowski (2018), in whose experiment Tytanit foliar application did not contribute to changes of calcium and phosphorus accumulation in potato tubers, leading to no diversification of their ratio.

5. Conclusions

1. Hybrid alfalfa had higher dry matter yields than red clover. The biggest differences between the species were in the second year of studies.
2. Different doses of Tytanit in different ways affected the yield. However, the largest dose of 0.6 dm^3 turned out to be the most effective. It contributed to a 38% increase in the yield of hybrid alfalfa and to a 31% increase in the red clover yield.
3. Individual doses of Tytanit in different ways affected accumulation of protein and crude fibre in the dry matter. Used at 0.4 and 0.6 dm^3 doses it increased the amounts of protein and crude fibre relative to control. The smallest dose had no significant effect on these parameters.
4. Tytanit did not improve dry matter digestibility, and there was no statistically significant variation as a result of its application.
5. Foliar application of the biostimulant resulted in an increase in the concentration of phosphorus, potassium, and magnesium in plant dry matter. High content of calcium in the plant species before Tytanit application increased further as a response to 0.2 and 0.4 dm^3 doses, with a slight increase in the ratio of K:(Ca + Mg) and an excessive growth of the Ca: P ratio, which reduced hybrid alfalfa and red clover nutritional values.
6. Thus, Tytanit doses used in the experiment significantly increased hybrid alfalfa and red clover yields, but the nutritional value of the plants did not improve. The results obtained in the experiment indicate the need to continue and develop research in this field. This contributed to the increase of knowledge on the effects of regulators based on active substances, including the mechanisms of their functioning in the cultivation of various plants.

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