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Effect of Auxin and Various Iron Concentrations on *Medicago* × *Varia* T. Martyn Yield and Forage Quality

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ABSTRACT

The aim of the four-year field experiment was to determine the effect of the foliar application of auxin, a synthetic growth hormone, used on its own and in combination with various iron concentrations on the yield and forage quality of *Medicago* × *varia* T. Martyn, the Kometa cultivar. The research was conducted in three replications at the experimental facility of Siedlce University (52°10′03"N; 22°17′24"E, Poland) between 2014 and 2017. The treatment combinations were as follows: K – control (distilled water); A – indole-3-butyric acid (IBA - synthetic auxin); A+F1 – auxin (indole-3 butyric acid) with iron (II) sulphate (IV) heptahydrate at 3% concentration; A+F2 – auxin (indole-3 butyric acid (IBA)) with iron (II) sulphate heptahydrate at 5% concentration; A+F3 – auxin (indole-3 butyric acid (IBA) with iron (II) sulphate heptahydrate at 7% concentration. Between 2014 and 2017 hybrid alfalfa was harvested at the beginning of the bud stage, three times a growing season. Each growth cycle, plants were sprayed once at the 9-leaf stage. During a harvest 0.5 kg of fresh matter was collected from each plot to determine the content of aboveground dry matter and to perform chemical analyses. The highest yield, 44% higher than for control plants, was noted on the plot treated with auxin applied together with iron sulphate at 5% concentration, and the lowest on the control plot. Foliar application of auxin with iron improved the quality of alfalfa forage, affecting the content of total and digestible protein and crude fibre and dry matter digestibility.

Keywords: growth hormone, iron, yield, total protein, crude fibre, *Medicago* × *varia* T. Martyn.

INTRODUCTION

Forage quality is determined by its nutritional value and nutritional effects on animal performance. According to many authors, dry roughage is essential in animals' winter diet as it is a basic factor maintaining the balance of the digestive system [Kavut and Avcioqlu, 2015]. Roughage quality is affected by such agricultural practices as quantity of fertilizers used on grassland and the timing of their application, harvesting technologies and the method of forage preparation for ruminant feeding [Brzóska and Śliwiński, 2011]. Harvesting alfalfa in the appropriate development stage (i.e. at the beginning of the bud period) has a significant impact on protein and crude fibre content. When the harvest is delayed, crude fibre content increases [Mosiman et al., 1998; Wilman 1997; Kallenbach et al., 2002]. Alfalfa hay is a source of protein, minerals and vitamins, especially carotene. Alfalfa forage harvested at full budding and flowering has a lower dry matter decomposition rate than harvested at another time [Ta and Faris, 1987].

Many studies have dealt with supplementing or replacing traditional mineral treatment of forage plants with growth regulators applied to leaves [Ozturk and Yildirim, 2013; Wani et al., 2017; Ivanova-Kovacheva et al., 2018; Ivanov and Nikolov, 2021; Mehmed and Enchew, 2020; Marinova et al., 2023]. Such solutions increase nutrient efficiency and ensure the stability of crop yields not only in stressful but also in optimal conditions [Bozhanska et al., 2017; Stoyanova et al., 2021]. Plant hormones are sensitive to a lack of balance, which means that any disturbance in the biosynthesis of one of them immediately activates or deactivates another hormone. The basic plant hormones are auxins, cytokinins, gibberellins, abscisic acid and ethylene. Auxins participate in all life processes of the plant, which is why they are considered to be the most important hormones. Among others, they take part in the formation of root buds, cell division and plant movement, also known as tropism [Matysiak and Adamczewski, 2009; Sosnowski et al., 2023]. Auxin and other growth regulators are most often used in floriculture and horticulture.

Plants react particularly negatively to abiotic stress, i.e. all kinds of environmental factors unfavourable for their growth and development. Some examples of stress are a long-term drought, increasingly common in many European countries, including Poland, soil salinity or nutrient deficiency. According to many studies, abiotic stress negatively affects plant development and the yield, which may be up to several times lower than the yield of plants subjected to biotic stress such as weed infestation, pathogens, etc. [Grossmann and Retzlaft, 1997; Kaydan et al., 2007].

The aim of the four-year field experiment was to determine the effect of foliar application of synthetic auxin in combination with iron sulphate various concentrations on the yield and protein and fibre content in the biomass of *Medicago × varia* T. Martyn.

MATERIALS AND METHODS

In the spring of 2014 the experiment was established at the experimental facility of the University of Siedlce (52°10′03”N; 22°17′24”E), in central-east Poland, on soil with granulometric composition of loamy sand with pH_{KCl} of 6.75. Carbon content in organic compounds determined by the oxidation-titration method was 37.0 g kg^{-1}, and total nitrogen determined by the Kiejdahl method was 1.75 g kg^{-1}. Determined by the Egner-Rhiem method, soil content of available phosphorus and potassium was moderate with 39.9 mg kg^{-1} and 128 mg kg^{-1}, respectively. The total content of macroelements in the soil was as follows (g kg^{-1}): P – 1.05; K – 1.00; Ca – 2.40; Mg – 1.25; S – 0.508; Na – 0.312. This content was determined using the ICP-AES method after dry soil mineralization at 450°C in a muffle furnace. In the experiment, hybrid alfalfa (*Medicago × varia* T. Martyn) of the Kometa cultivar was used. The seeding rate was adopted according to the standards developed by the Institute for Land Reclamation and Grassland Farming in Falenty [Jankowski et al., 2005]. The experimental field was divided into plots, each with an area of 3 m^2. The experiment was replicated three times and arranged in a completely randomized design, with the following treatment combinations:

- K – control (without treatment);
- A – indole-3-butyric acid (IBA – synthetic auxin);
- A + F1– auxin (indole-3 butyric acid (IBA)) + iron(II) sulphate (IV) heptahydrate with a concentration of 3%;
- A + F2 – auxin (indole-3 butyric acid (IBA)) + iron(II) sulphate heptahydrate with a concentration of 5 %;
- A+ F3 – auxin (indole-3 butyric acid (IBA) + iron(II) sulphate heptahydrate with a concentration of 7%.

For a single spraying, 0.5 l of solution with a regulator concentration of 35 mg l^{-1} was used to cover plants with the liquid completely. Control plants were sprayed with the same amount of distilled water. During each growth cycle plants were sprayed once, during the 9-leaf stage. Each year between 2014 and 2017, hybrid alfalfa was harvested three times, at the beginning of the bud stage. During a harvest, 0.5 kg of fresh matter was collected from each plot to determine the weight of aboveground dry matter and to perform chemical analyses. The yield of hybrid alfalfa dry matter was determined by drying the sample until constant weight at 105°C. The content of total protein and crude fibre was determined by near-infrared reflection spectroscopy (NIRS) using the NIRFex N-500 apparatus.

The nutritional value of grassland forage was determined based on protein and crude fibre content [Pawlak, 1988; Ostrowski, 1990]. Thus, digestible protein content (Y) was calculated using the formula:

$$Y = -29.78 + 9.56 \times b \text{ (g kg}^{-1}\text{DM)} \quad (1)$$

where: b – total protein content in % DM.

Digestible fibre content (S) was also calculated according to the following formula:

$$S = 103 - 1.2 \times a \text{ (\% DM)} \quad (2)$$

where: a – crude fibre content in % DM.

Meteorological data from between 2014 and 2017 were provided by the Institute of Meteorology and Water Management, National Research Institute (PIB) in Warsaw, the Hydrological and Meteorological Station in Siedlce. The results were statistically processed, and the differences between means were assessed using analysis of variance. Statistica Version 10.00 [StatSoft, inc. 2011], was used for calculations, and in the case of significant differences, the value of $NIR_{0.05}$ was calculated according to Tukey's test. Pearson linear correlation coefficients were calculated to assess the relationship between the hybrid alfalfa dry matter yield on the one hand and the content of total and digestible protein and crude fibre and dry matter digestibility on the other ($p \leq 0.05$).

RESULTS AND DISCUSSION

During *Medicago* × *varia* T. Martyn full use (2014-2017) optimal thermal conditions prevailed, with the average temperature close to the multiannual one (Table 1), except for July 2014 and August 2015 when it was much higher, exceeding 20°C. The average precipitation across the four years was similar to the multiannual rainfall, with the exception of July and August, with much lower values. There were very wet and very dry periods throughout the experiment. In the first year (2014), a very small amount of precipitation was recorded in October. In 2015, May, September and October were quite wet, while the other months of the growing period were very or extremely dry. In the third year (2016), optimal rainfall and air temperature were recorded in September, May was very wet, while June, July and August, the most important months for the growth and development of plants, were quite dry, dry and extremely dry. In 2017, soil moisture conditions were variable; the beginning and end of the growing season were very wet, while precipitation in July was much lower than the multiannual average.

The dry matter yield of hybrid alfalfa in subsequent years varied across treatment combinations and years of research (Table 2). Each growing period, a significantly lower biomass yield was recorded on the control plot than on plots with fertilizer treatments. On the control plot the annual yield, average of four years, was 9.49 Mg ha^{-1}. The highest average yield of 13.70 mg ha^{-1}, 44% higher than for control plants, was recorded in response to auxin used together with 5% iron sulphate (A+Fe2). On the other fertilized plots, a yield increase compared to control was also significant, and amounted to over 19% in response to auxin (A) used on its own, 32% in response to auxin with 3% iron sulphate (A+Fe1), and 36% in response to auxin with iron sulphate at a 5% concentration (A+Fe3). Nowak and Wróbel [2010a, b] recorded a significant 34% increase in a soybean yield in response to auxin. An increase in the yield of peas treated with indole-3-butyric acid was observed by Reinecke [1999]. Many other authors confirm the beneficial effects of synthetic auxin on plant growth and development [Karaguzel et al., 1999; Czapla et al., 2003; Jamil et al., 2016]. Additionally, Marinova et al. [2023] report an increase in alfalfa yield after application

Table 1. Average monthly temperature (°C) and rainfall (mm)

Year	Month							
	April	May	June	July	August	September	October	Mean
Temperature (°C)								
2014	9.7	13.7	15.1	20.5	17.8	13.7	8.4	14.1
2015	8.2	12.3	16.5	18.7	21.0	14.5	6.5	13.9
2016	7.9	11.1	18.7	18.4	19.3	14.9	10.6	14.4
2017	8.3	13.9	17.8	16.9	18.4	13.9	9.0	14.0
Mean	8.5	12.8	17.0	18.6	19.1	14.3	8.6	14.1
Multiannual mean of 2003-2013	8.5	14.0	17.4	19.8	18.9	13.2	7.9	14.2
Rainfall (mm)								
2014	39.5	79.5	74.2	37.5	105.7	26.3	3.0	52.2
2015	30.3	100.2	43.3	62.6	11.9	77.1	39.0	52.1
2016	40.2	74.2	40.2	50.2	36.1	29.4	34.1	43.5
2017	59.6	49.5	57.9	23.6	54.7	80.1	53.0	54.1
Mean	42.4	75.9	53.9	43.5	52.1	53.3	32.3	50.5
Multiannual mean of 2003-2013	33.0	52.0	52.0	65.0	56.0	48.0	28.0	47.7

Table 2. Dry matter yield of hybrid alfalfa (Mg ha^{-1}) in subsequent years (total of three harvests)

Treatment	Year 1	Year 2	Year 3	Year 4	Mean
Control	8.91	9.52	9.04	10.50	9.49
A	10.42	11.56	12.01	11.42	11.35
A +Fe1	12.91	12.63	12.05	12.50	12.52
A +Fe2	13.85	12.83	14.19	13.91	13.70
A +Fe3	12.42	13.01	13.62	12.63	12.92
Mean	11.70	11.91	12.18	12.19	11.99
$LSD_{0.05}$ for: A – treatment A=0.518; B- years; B=0.435; B/A; A/B – interaction B/A=0.972; A/B=1.04					

Note: A – indole-3-butyric acid (IBA – synthetic auxin), A + F1-auxin (indole-3 butyric acid (IBA)) + iron(II) sulphate heptahydrate with a concentration of 3%, A+ F2- auxin (indole-3 butyric acid (IBA)) + iron(II) sulphate heptahydrate with a concentration of 5%, A+ F3- auxin (indole-3 butyric acid (IBA) + iron(II) sulphate heptahydrate with a concentration of 7%.

of auxin-enriched vermicompost extracts. In the first year, the dry matter yield of hybrid alfalfa was significantly the lowest, and amounted to an average of 11.70 Mg ha^{-1}. In the third and fourth years, the highest average yields were recorded, with 12.18 and 12.19 Mg ha^{-1}, respectively.

Protein content in hybrid alfalfa dry matter ranged from 19.05 to 23.01% (Table 3). Compared to control plants and to those treated with auxin on its own, auxin in combination with iron sulphate resulted in its significant increase. Significantly the highest protein content of 22.55% and 22.18% was recorded on plots where auxin and higher concentrations of iron (A + Fe2 and A + Fe3) were applied. This parameter did not vary significantly across the years of research. The lowest protein content was in forage collected in the first year, with an average of 20.96%. In the following years, the average protein content was as follows: 21.51, 21.39 and 21.08%. In forage crops it usually ranges from 10–12 to 16–18% DM [Brzóśka and Śliwiński 2011]. According to many authors [Mosiman et al., 1998; Wilman, 1997; Kallenbah et al., 2002], protein content in alfalfa forage is significantly affected by the harvest date, and with a delay of harvest the value of this parameter decreases.

When feeding animals with roughage, high protein digestibility is important. According to Ostrowski [1991], differences between total and digestible protein content may be a result of higher nitrogen doses. The content of alfalfa digestible protein was similar to the content of total protein (Table 4). The highest content of digestible protein was noted in response to auxin and higher concentrations of iron (A + Fe2 and A + Fe3), with 185.97 and 182.21 g kg^{-1} DM, respectively. Across years of research and treatments, the average digestible protein content in alfalfa was 173.28 g kg^{-1} DM. In permanent grassland hay Kolczarek et al. [2008] noted values ranging from 99 to 204.1 g kg^{-1} DM. In the present experiment years of research did not have a significant impact on protein content. The lowest average protein digestibility was recorded in the first year. This might have been due to an unfavourable

Table 3. Protein content (%) in the dry matter of hybrid alfalfa in subsequent years

Treatment	Year 1	Year 2	Year 3	Year 4	Mean
Control	19.05	20.35	20.30	19.49	19.80
A	19.22	19.84	20.60	20.75	20.10
A +Fe1	21.96	21.80	21.53	20.95	21.56
A +Fe2	22.96	23.01	22.58	21.63	22.55
A +Fe3	21.62	22.54	21.96	22.58	22.18
Mean	20.96	21.51	21.39	21.08	21.24
$LSD_{0.05}$ for: A – treatment A=0.696; B- years B=NS; B/A; A/B – interaction B/A=1.31; A/B=1.39					

Note: NS – not significant, see Table 2 for legend.

Table 4. Digestible protein content (g kg^{-1}DM) in hybrid alfalfa in subsequent years

Treatment	Year 1	Year 2	Year 3	Year 4	Mean
Control	152.34	164.77	164.29	156.54	159.49
A	154.00	159.89	167.16	168.59	162.41
A +Fe1	180.16	178.63	176.05	170.50	176.34
A +Fe2	189.72	191.06	186.08	177.00	185.97
A +Fe3	176.91	185.70	180.16	186.08	182.21
Mean	170.63	176.01	174.75	171.74	173.28
$LSD_{0.05}$ for: A – treatment A= 11.18 B- years B= NS B/A; A/B – interaction B/A= NS; A/B= NS					

Note: NS – not significant difference, see Table 2 for legend.

distribution of precipitation during that growing season (Table 1). Across the experimental years crude fibre content in hybrid alfalfa dry matter ranged from 22.40 to 28.40% (Table 5). On average, the highest value was found on the control plot (26.73%) and on that treated with foliar application of auxin with 3% iron sulphate (A+ Fe1) (26.15%). However, crude fibre content was significantly lower in response to auxin applied together with the highest amounts of iron (A + Fe2 and A + Fe3), with 24.63 and 24.33%, respectively. Crude fibre content significantly varied not only across fertilizer treatments but also across growing seasons; the lowest value was recorded in the first year (24.70%) and the highest in the fourth (25.94%). Some authors argue that alfalfa fibre content depends not only on the developmental stage, but also on the height of stems [Hintz and Albrecht, 1991]. Similarly, Andrzejewska et al. [2013] report that the developmental stage of alfalfa is not a sufficient indicator of forage quality because plant height can equally or more determine plant chemical composition.

The level of total digestibility ranged from 68.92 to 76.12% (Table 6). According to Preś [1977], the digestibility of cattle forage should not be lower than 65–67%. Forage with low digestibility is stuck in the rumen. In the present experiment, much higher values were recorded than the recommended minimum amount. The highest digestibility (73.81%) was found in alfalfa from the plot where auxin was used in combination with iron sulphate at a concentration of 7%, and the lowest from the control plot (70.93% on average). Foliar application of auxin (A) on its own increased dry matter digestibility, compared to plants treated with auxin in combination with the lowest concentration of iron (A + Fe1).

Years of research significantly affected the digestibility of hybrid alfalfa dry matter, which continually decreased throughout the experiment. In the first year, it averaged 73.35%, 72.47% in the second, 71.95% in the third and 71.87% in the fourth.

Linear correlation coefficients were used to determine the relationship between the dry matter yield of hybrid alfalfa on the one hand and the content of total and digestible protein, crude fibre and dry matter on the other (Table 7). A statistically significant positive relationship was noted

Table 5. Crude fibre content (%) in the dry matter of hybrid alfalfa in subsequent years

Treatment	Year 1	Year 2	Year 3	Year 4	Mean
Control	26.79	25.84	26.47	27.79	26.73
A	25.70	27.22	25.84	23.84	25.65
A +Fe1	23.79	25.20	27.20	28.40	26.15
A +Fe2	24.84	23.94	26.49	23.25	24.63
A +Fe3	22.40	25.01	23.44	26.44	24.33
Mean	24.70	25.44	25.89	25.94	25.49
$LSD_{0.05}$ for: A – treatment A= 0.975 B- years B= 0.818 B/A; A/B – interaction B/A= 1.83; A/B= 1.95					

Note: See Table 2 for legend.

Table 6. Dry matter digestibility (%) of hybrid alfalfa forage in subsequent years

Treatment	Year 1	Year 2	Year 3	Year 4	Mean
Control	70.85	71.99	71.24	69.65	70.93
A	72.16	70.34	71.99	74.39	72.22
A +Fe1	74.45	72.76	70.36	68.92	71.62
A +Fe2	73.19	74.27	71.30	75.10	73.47
A +Fe3	76.12	72.99	74.87	71.27	73.81
Mean	73.35	72.47	71.95	71.87	72.41
$LSD_{0.05}$ for: A – treatment A= 0.522 B- years B= 0.438 B/A; A/B – interaction B/A= 0.979; A/B= 1.04					

Note: See Table 2 for legend.

Table 7. Linear correlation coefficient between the dry matter yield of hybrid alfalfa and the content of total protein, digestible protein, crude fibre and dry matter digestibility

	Dry matter yield	Total protein	Digestible protein	Crude fibre	Dry matter digestibility
Dry matter yield	1.00				
Total protein	0.829*	1.00			
Digestible protein	0.827*	0.999*	1.00		
Crude fibre	-0.413	-0.447*	-0.449*	1.00	
Dry matter digestibility	0.417	-0.450*	0.452*	-0.999*	1.00

Note: p≤0.05, * Significant correlation.

between the content of total protein (r = 0.829) and digestible protein (r = 0.827) in biomass, which means that when the former increased, the latter increased too. A significant positive relationship was also found between total and digestible protein content in alfalfa forage. On the other hand, there was a negative correlation between total protein content on the one hand and crude fibre content and dry matter digestibility on the other. There was a significant negative relationship between digestible protein content and crude fibre content of alfalfa forage, and a significantly positive relationship between digestible protein content and dry matter digestibility. There was a highly significant negative relationship between crude fibre content and dry matter digestibility (r = -0.999). This means that the more crude fibre in the forage, the lower its digestibility. The main criterion for forage quality is high content of total protein and low content of crude fibre.

CONCLUSIONS

Compared to control, the yield of *Medicago × varia* T. Martyn dry matter significantly increased in response to foliar application of auxin on its own or in combination with iron sulphate. The highest value was noted for plants treated with auxin applied together with 5% iron sulphate, which meant a 44% increase compared to control. Taking into account the content of total protein and crude fibre as well as dry matter digestibility, the application of auxin in combination with higher concentrations of iron sulphate resulted in forage of the best quality. Thus, foliar application of auxin in combination with iron sulphate has promising results and can be recommended in the production of hybrid alfalfa forage.

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