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Effect of a Micronutrient Fertilizer and Fungicide on the Germination of Perennial Ryegrass Seeds (*Lolium perenne* L.) in Field Conditions

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Abstract: The aim of this research was to determine the effect of micronutrients and a fungicide on the germination of perennial ryegrass seeds. The experiment was conducted between 2016 and 2018 in Blizocin (51°22′ N, 17°09′ E), Poland. The first experimental factor was InnoFert Mikro, a micronutrient fertilizer used at doses of 0.5 and 1 L·ha^{−1}, while the second factor was the Soprano 125 S.C. fungicide used at the same doses. From each of the plots, 100 seeds were collected and placed on a Petri dish. Then, every day for 14 days, the plant germination was observed. The highest germination capacity of 95.3% was recorded for seeds from plots where combined full doses of the experimental factors were applied. This figure was 2% higher than for the control sample. The germination speed of the seeds from the above plot, determined by Maguire’s index, was the highest out of the other seed groups, although the seeds from the above plot had the longest average germination time. It was also noted that by increasing the dose of micronutrient fertilizer, the germination speed also increased. In the case of the fungicide, half a dose had a positive effect on germination, but increasing the dose to 1 L·ha^{−1} resulted in fewer germinated plants when compared to the control sample.

Keywords: grass; two-factor experiment; germination capacity; Pieper’s index; Maguire’s index

1. Introduction

One of the most commonly grown and valuable grass species of temperate climates, used to plant new meadows and pastures, lawns, and sports grounds, is perennial ryegrass [1–5]. It produces high yields and is easy to plant and grow even on heavy soils and on wetlands [6,7]. There has been a significant increase in its use in agriculture as well, and it is now considered to be economically the most important grass species in Europe, New Zealand, and in some regions of Japan, Australia, South Africa, and South America [8]. Currently, perennial ryegrass is grown for forage, green fertilizer, and energy purposes, but seed production is still dominant, and it is a promising and cost-effective alternative to other grass species. In addition, the climatic conditions of Poland and neighboring European countries are conducive to the establishment of perennial ryegrass fields [9–11]. Since 2000, about 83,660 Mg of ryegrass seeds per year have been produced on average in Europe and 209,674 Mg in the world [3].

From an economic point of view, a high yield of seeds is most desirable for farmers who decide to grow the plant for seed production. However, the yield depends on several complex factors, including farming techniques, the way the crop is grown, as well as environmental conditions [12]. Mineral fertilizers and crop protection products are widely used to increase its production. While there have been many studies on mineral fertilizer use, including nitrogen, potassium, and phosphorus doses [13–16], there is still a lack of literature describing the effect of micronutrient application on seed production levels. These fertilizers can be used to increase yields and production efficiency, especially when the application of conventional Nitrogen-Phosphorus-Potassium (NPK) products is inefficient [17–19]. In the case of perennial ryegrass, the use of fungicides is an equally important element, as many fungal diseases can lead to high crop losses [20]. Studies have shown that the application of fungicides is economically profitable and that an increase in the yield of perennial ryegrass seeds resulting from their application is in the range of 180–920 kg·ha^{−1} [21].

Seed germination is an important step in plant growth and development. What seeds need in order to germinate is the right ecological environment [22,23]. Many studies have focused on determining the significance of the impact of environmental conditions on the germination capacity [24,25]. It has been proven that the main factors determining the development and maturation of seeds and, consequently, their germination capacity, are temperature [26,27], water content [28], or the length of the day [29].

In view of an increasing interest in this grass species, it appears justified to study the effect of new methods in the production of its seeds. That is why the aim of this paper is to investigate the effect of a micronutrient fertilizer in combination with a fungicide on the germination capacity of perennial ryegrass.

2. Materials and Methods

2.1. Experimental Field

The studies were conducted between 2016 and 2018 in Blizocin (51°22′ N, 17°09′ E) near Wrocław, Poland, on class Iva soil according to the Polish soil classification. Melfrost, a forage variety of perennial ryegrass with a seeding rate of 20 kg·ha^{−1}, was used in the research. With 1.5 m × 20 m plots, each with an area of 30 m², this was a two-factor experiment, with four replications and a split-plot design.

The first experimental factor (A) was InnoFert Mikro (produced by ADOB Company, Poznań, Poland), a micronutrient fertilizer containing 75 g·kg^{−1} Mn, 52 g·kg^{−1} Zn, 10 g·kg^{−1} Cu, 5 g·kg^{−1} B, and 2 g·kg^{−1} Mo. The experimental plots were treated as follows: A0—control; A1—half a dose of micronutrients (0.5 kg of fertilizer per ha) with Mn 37.5 g, Zn 26 g, Cu 5 g, B 2.5 g, Mo 1 g; A2—a full dose of micronutrients (1 kg of fertilizer per ha, as recommended by the manufacturer). The fertilizer was applied to the leaves using a hand sprayer.

The second experimental factor (B) was Soprano 125 S.C., a fungicide with 125 g·L^{−1} of epoxyconazole as an active substance. The experimental plots were treated as follows: B0—control; B1—half a dose of fungicide (0.5 L·ha^{−1} with 62.5 g of epoxyconazole); B2—a full dose of fungicide (1 L·ha^{−1}, with 125 g of epoxyconazole). The fungicide was applied using a hand sprayer. Table 1 presents the experimental plots with all the treatment combinations.

Table 1. Details of the application.

Substance	Time of Application	Growth Stage	Rate
Micronutrient	1st decade of April	Full tillering—2nd node	Single rate: 1 kg·ha ^{−1}
Fungicide	1st decade of April	Full tillering—2nd node	Single rate: 1 L·ha ^{−1}

The harvest was realized in the first decade of August using a plot-harvester with an operational width of 1.5 m. The germination experiment was conducted once, and it was started in the first decade of December. The seeds chosen for the experiment were described using 1000 seeds' weight. This was conducted using an LN-S-50A seeds counter, after which the seeds were weighed on a WPS 4000/C/2

(produced by RADWAG Company, Radom, Poland) weighing scale—the accuracy was equal to 0.01 g, with a measuring range: 0–4000 g.

2.2. Weather Conditions

During the experiment, weather conditions were unfavorable for the growth and development of perennial ryegrass. Between 2016 and 2018 during the grass growing season (April–August), the total amount of precipitation was 280.4 mm, 442.0 mm, and 260.6 mm, respectively. The average annual air temperature was 9.9 °C, 10.0 °C, and 10.8 °C, but between April and August it was 16.3 °C, 15.8 °C, and 18.2 °C (Table 2).

Table 2. Monthly precipitation and average daily air temperature.

Month	Rainfall [mm]				Mean Temperature [°C]			
	2016	2017	2018	2016–2018	2016	2017	2018	2016–2018
January	56.2	13.5	20.6	30.1	−1.1	−3.2	2.9	−0.5
February	27.7	27.5	2.8	19.3	3.9	1.2	−2.4	2.7
March	56.2	35.0	26.5	39.2	4.3	6.9	1.2	4.1
April	27.7	63.8	24.6	38.7	8.8	8.3	13.9	10.3
May	26.4	40.2	49.4	38.7	15.3	14.6	17.1	15.7
June	59.6	65.2	51.1	58.6	19.0	19.1	19.5	19.2
July	105.0	142.6	72.9	106.8	19.9	19.6	20.8	20.1
August	22.6	64.1	11.4	32.7	18.5	19.9	21.7	20.0
September	39.1	66.1	51.2	52.1	16.5	13.5	16.1	15.4
October	87.5	71.4	46.1	68.3	8.5	11.1	10.4	10.0
November	44.0	34.8	12.5	30.4	3.7	5.7	5.3	4.9
December	37.2	27.1	41.7	35.3	1.6	3.0	2.6	2.4
Total (April–September)					Mean (April–September)			
280.4					16.3			
442.0					15.8			
260.6					18.2			
327.7					16.8			
Total					Mean			
589.2					9.9			
651.3					10.0			
410.8					10.8			
724.3					10.2			

2.3. Germination Capacity Determination

100 seeds collected from each plot were put in a Petri dish. Then, for 14 days, the germination was assessed on a daily basis by counting the number of germinated plants. Seeds that developed roots were removed from the dish. On the basis of the results, an analysis of the germination capacity was carried out, including parameters such as the mean germination time (Pieper's index), germination speed (Maguire's index), and the average number of seeds germinated during one day (Kotowski's index).

Pieper's index denoting the mean germination time was determined as the quotient of the sum of the products of the numbers of germinated seeds on a given day and the number of days from sowing to the removal of the seeds from the dish to the sum of those seeds, according to the formula:

$$\text{Pieper's index} = \frac{(m_1d_1 + m_2d_2 + \dots m_nd_n)}{(m_1 + m_2 + \dots m_n)} \quad (1)$$

where:

m—the number of germinated seeds on a given day,

d—the number of days from sowing to the removal of the seeds from the dish.

Maguire's index denoting the germination speed was determined as the sum of the quotients of the number of germinated seeds on a given day and the number of days from sowing to the removal of the seeds from the dish, according to the formula:

$$\text{Maguire's index} = \frac{m_1}{d_1} + \frac{m_2}{d_2} + \dots + \frac{m_n}{d_n} \quad (2)$$

where:

m—the number of germinated seeds on a given day,

d—the number of days from sowing to the removal of the seeds from the dish.

Kotowski's average daytime seed ratio was determined as the quotient of the sum of the germinated seeds and the sum of the products of the number of germinated seeds on a given day and the number of days from sowing to the removal of seeds from the dish, according to the formula:

$$\text{Kotowski's index} = \frac{(m_1 + m_2 + \dots + m_n)}{(m_1 d_1 + m_2 d_2 + \dots + m_n d_n)} \quad (3)$$

where:

m—the number of germinated seeds on a given day,

d—the number of days from sowing to the removal of the seeds from the dish.

2.4. Statistical Data Analysis

The obtained results were confirmed using a statistical analysis. It was conducted in Statistica 12.5 software (serial number JPZP703B482817AR-H, license for Wroclaw Univeristy of Environmental and Life Sciences). To determine the significance of the factors, an analysis for variance was used (level of significance $\alpha = 0.05$). The differences between the levels of factors were determined using an LSD Fisher test.

3. Results

Table 3 shows the values of 1000 seeds at different rates of the microelement and fungicide. The highest values (3.65 g) were observed for the treatments A2-B0 and A2-B1, while the lowest value (3.53) concerned the combination A1-B1. However, it should be emphasized that the differences between the values at the treatments were small and statistically insignificant.

Table 3. Effect of micronutrients and fungicide on 1000 seeds' weight.

Treatment		Factor A [g]			Mean
		Control (A0)	0.5 kg of Microelement Fertilizer (A1)	1 kg of Microelement Fertilizer (A2)	
Factor B [g]	Control (B0)	3.65 ¹	3.60 ¹	3.68 ¹	3.64
	0.5 L of fungicide (B1) ¹	3.65 ¹	3.53 ¹	3.68 ¹	3.62
	1 L of fungicide (B2) ¹	3.58 ¹	3.65 ¹	3.65 ¹	3.63
	Mean	3.63	3.59	3.67	3.63

¹ A = NS; B = NS; A × B = NS. NS—not significant.

The effect of the micronutrient fertilizer and fungicide on the number of seedlings that sprouted from perennial ryegrass seeds after a certain number of days is presented in Table 4. Some seedlings emerged on the dishes as early as two days after germination started. However, a significant increase in the number of sprouting roots was noted only a day later. On average, after three days the germination

capacity was 24%. Although observations were made over 14 days, the ultimate number of germinated plants in all treatments was recorded earlier, after 10 days. After this period, no new seedlings emerged. When counted at the end of the observations, the greatest number of germinated seeds was from the plot with a full dose of the micronutrient fertilizer combined with a full fungicide dose (A2 × B2). On average, the germination capacity for this combination was 95.3%, which was 2% higher than for seeds from control (A0 × B0). A high value was also recorded for the combination of half of the first factor dose with half of the second factor dose (95.0%). In contrast, the lowest germination capacity was for seeds from the plots with a full dose of the fungicide applied on its own (93.0%). When relating the effect of the two factors to the control, it was found that as the dose of the micronutrient fertilizer (A) increased, the number of germinated plants also increased. The situation was slightly different when the fungicide (B) was used. Half of its dose increased the germination capacity by 1.5% relative to the control. However, increasing the dose further resulted in a 0.3% decrease. The average share of germinated plants from all treatments was 94.2%.

Table 4. Effect of the micronutrient fertilizer and fungicide on the number of germinated seeds after a given number of days.

Treatment		Factor A			Mean
		Control (A0)	0.5 kg of Microelement Fertilizer (A1)	1 kg of Microelement Fertilizer (A2)	
Factor B (after 1 day)	Control (B0)	0.0 ¹	0.0 ¹	0.0 ¹	0.0
	0.5 L of fungicide (B1)	0.0 ¹	0.0 ¹	0.0 ¹	0.0
	1 L of fungicide (B2)	0.0 ¹	0.0 ¹	0.0 ¹	0.0
	Mean	0.0	0.0	0.0	0.0
Factor B (after 5 days)	Control (B0)	87.3 ^{1,2}	79.5 ¹	85.0 ¹	83.9
	0.5 L of fungicide (B1)	80.3 ¹	81.3 ^{1,2}	83.0 ^{1,2}	81.5
	1 L of fungicide (B2)	76.8 ^{1,2}	78.5 ¹	86.5 ^{1,2}	80.6
	Mean	81.5	79.8	84.8	82.0
Factor B (after 10 days)	Control (B0)	93.3 ¹	93.5 ¹	94.0 ¹	93.6
	0.5 L of fungicide (B1)	94.8 ¹	95.0 ¹	94.8 ¹	94.8
	1 L of fungicide (B2)	93.0 ¹	94.5 ¹	95.3 ¹	94.3
	Mean	93.7	94.3	94.7	94.2
Factor B (after 14 days)	Control (B0)	93.3 ¹	93.5 ¹	94.0 ¹	93.6
	0.5 L of fungicide	94.8 ¹	95.0 ¹	94.8 ¹	94.8
	1 L of fungicide	93.0 ¹	94.5 ¹	95.3 ¹	94.3
	Mean	93.7	94.3	94.7	94.2

¹ A = NS; B = NS; A × B = NS. ² LSD α A = 0.05 = 1.4; B = NS; A × B = NS. NS—not significant.

Table 5 shows the effect of the micronutrient fertilizer and fungicide on Maguire's germination speed index for each combination. The higher the value of the index was, the greater was the ability of seeds to germinate quickly [30]. By far, the highest germination speed index (286.2) was recorded for seeds from plots with combined full doses of the micronutrient fertilizer and fungicide (A2 × B2). On the other hand, the smallest values were recorded when each of the factors was applied on its own at a full dose. For the InnoFert Mikro fertilizer it was 275.3 (A2 × B0), and for Soprano 125 S.C. it was slightly less, 275.2 (A0 × B2). By increasing the doses of micronutrients (A0 × B0; A1 × B0; A2 × B0), the germination speed decreased. In the case of the fungicide, half a dose increased the germination speed index by 0.9 (A0 × B1), but an increase of its dose to 1 L resulted in a significant

decrease in the index value (by 5.6). The mean value of the germination speed index was 281.2 for all fertilizer combinations.

Table 5. Effect of micronutrients and fungicide on Maguire’s germination speed index.

Treatment		Factor A			Mean
		Control (A0)	0.5 kg of Microelement Fertilizer (A1)	1 kg of Microelement Fertilizer (A2)	
Factor B	Control (B0)	280.8 ¹	278.3 ¹	275.3 ¹	280.3
	0.5 L of fungicide (B1)	281.7 ¹	283.1 ¹	280.3 ¹	282.8
	1 L of fungicide (B2)	275.2 ¹	283.6 ¹	286.2 ¹	280.6
	Mean	279.2	280.6	283.8	281.2

¹ A = NS; B = NS; A × B = NS. NS—not significant.

Table 6 presents the effect of the micronutrients and fungicide on Pieper’s index, reflecting the mean germination time for each combination. It ranged from 54.4 to 59.5, with an average of 56.9 for all combinations. The highest value was from the plot with a full dose of the fungicide combined with a full dose of the micronutrient fertilizer (A2 × B2), and the lowest was for seeds treated with a full dose of fungicide on its own (A0 × B2). As the fungicide dose increased to 1 L·ha^{−1}, Pieper’s index decreased. On the other hand, the use of half a dose of the fertilizer (A1 × B0) resulted in a significant decrease in the mean germination time of the perennial ryegrass seeds (by 3.5), but increasing the dose to 1 L (A2 × B0) resulted in its relatively high increase (by 2.5). The lower the value of Pieper’s index was, the higher was the seed vigor and the faster was the germination. On the other hand, a high value of the index indicated that the germination was more stretched over time [31].

Table 6. Effect of different combinations of the micronutrient fertilizer and fungicide doses on Pieper’s germination time index.

Treatment		Factor A			Mean
		Control (A0)	0.5 kg of Micronutrient Fertilizer (A1)	1 kg of Micronutrient Fertilizer (A2)	
Factor B	Control (B0)	58.8 ¹	55.2 ¹	57.8 ¹	57.3
	0.5 L of fungicide (B1)	56.1 ¹	56.9 ¹	58.1 ¹	57.0
	1 L of fungicide (B2)	54.4 ¹	55.3 ¹	59.5 ¹	56.4
	Mean	56.4	55.8	58.4	56.9

¹ A = NS; B = NS; A × B = NS. NS—not significant.

Table 7 shows the effect of the micronutrient fertilizer and fungicide on the values of Kotowski’s index, with an average number of germinated seeds per day for each combination. It ranged from 0.166 to 0.172, with an average value of 0.169 for all combinations. Kotowski’s index was the highest for seeds from plants treated with a full dose of the fungicide (A0 × B2) and when in combination with a lower dose of the micronutrient fertilizer (A1 × B2). In turn, its lowest value was recorded for seeds from the control (A0 × B0). It was found that as the dose of the fungicide increased, Kotowski’s index increased. The use of the micronutrient fertilizer on its own had a positive effect on the index, but the application of half a dose (A1 × B0) resulted in a higher average number of sprouted seeds per day than when using a full dose (A2 × B0).

Table 7. Effect of the micronutrient fertilizer and fungicide on Kotowski's index, denoting the mean number of germinated seeds per day.

Treatment		Factor A			Mean
		Control (A0)	0.5 kg of Micronutrient Fertilizer (A1)	1 kg of Micronutrient Fertilizer (A2)	
Factor B	Control (B0)	0.166 ¹	0.171 ¹	0.168 ¹	0.168
	0.5 L of fungicide (B1)	0.170 ¹	0.170 ¹	0.168 ¹	0.169
	1 L of fungicide (B2)	0.172 ¹	0.172 ¹	0.167 ¹	0.170
	Mean	0.169	0.171	0.167	0.169

¹ A = NS; B = NS; A × B = NS. NS—not significant.

4. Discussion

The subject of the present studies was the effect of a micronutrient fertilizer and fungicide on the perennial ryegrass germination capacity. Several literature items describe the effects of these agents on other plants. Dhanamanjuri et al. [32] demonstrated that the use of fungicides may have a stimulating effect on seed germination. Several other authors [33,34] also came to similar conclusions. The use of these plant protection products in the production of perennial ryegrass seeds appears crucial in view of the number of potential diseases that may lead to a significant decline in the seed's yield and quality. Tietjen [35] shows that the use of chemical compounds with epoxiconazol can stimulate the plants positively. One of the most dangerous fungal diseases that must be controlled is stem rust, caused by *Puccinia graminis* spp. *graminicola* Z. Urb. Owing to its high ability to spread quickly, it can decrease the yield by up to 50–80%. Some other particularly dangerous diseases that can cause significant crop yield losses are leaf rust caused by *Puccinia* spp. and snow mold caused by *Microdochium nivale* (Fr) Samulem&Hallett. Affected plants produce a decreased yield that is up to 50% lower [36]. In view of the many challenges of perennial ryegrass protection, the use of fungicides is one of the key tools for guaranteeing stable yields.

Skinder and Gałczyński [37] believe that micronutrient fertilizer improves germination capacity and energy. Based on a review of many studies, Mondal and Bose [38] conclude that micronutrient fertilizer application is a promising and cost-effective method that affects seed quality and development. The microelements applied in the present research (Mn, Zn, Cu, B, Mo) are among the nutrients that have the greatest impact on the regulation of biochemical processes in plants during the growing season [39]. Micronutrient topdressing application to leaves is used to achieve the highest possible yield or to make up for deficiencies in the soil. In view of an increasing intensification of crop production, including a need to ensure the economic profitability of perennial ryegrass growth, micronutrient products are more widely used.

Previous studies have mainly dealt with the effect of the temperature, length of the day, and salinity level on the germination of perennial ryegrass seeds [40]. Alkaline stress [41], the presence of seminal fungi, and the correlation between the basic physical properties of the seeds, with their diameter, shape factor, spherical index, and density [42], have also been studied. It can therefore be noted that in recent years a number of studies have been undertaken to determine the key factors affecting perennial ryegrass germination, given the importance of its seed production in many regions of the world. However, the question of how it is affected by the combined use of micronutrient fertilizers and fungicides has not been proven.

This experiment demonstrated that combining fungus control with foliar micronutrient application had a positive effect on the germination capacity of perennial ryegrass. Depending on the combination, an increase in the number of germinated seeds relative to the control ranged from 1.2 to 2.0%, with the results showing that the treatment was extremely effective. The application of the micronutrient fertilizer on its own also increased the germination capacity, unlocking the full potential of that species.

On the other hand, the fungicide used at a full dose on its own decreased the germination capacity when compared to the control. In addition, Pieper's index was highly differentiated, but there was no significant delay or acceleration of plant germination due to the treatment of the plants by the first and second factors. Although the mean germination time was the longest for combined full doses of the fungicide and micronutrient fertilizer, Maguire's index indicated, with its high value for this combination (the highest out of all the treatments), that the grass responded to this treatment with a faster germination of its seeds. The use of fungicide in combination with micronutrient fertilizer can therefore lead to an effective improvement in the quality of seeds, increasing their germination capacity, and thereby potentially contributing to economic benefits in their production.

5. Conclusions

- The fungicide in combination with micronutrient fertilizer had a positive effect on the germination of perennial ryegrass seeds. For the combined application of their full doses ($A2 \times B2$, $1 \text{ L} \cdot \text{ha}^{-1}$ each), 95.3% of the seeds germinated, which was 2% more than for the control;
- Maguire's index, with its high value for the combined full doses of the first and second factors ($A2 \times B2$), indicated the ability of the seeds to germinate quickly;
- The germination time increased for full combined doses of the fertilizer and fungicide ($A2 \times B2$);
- The application of micronutrients improved the germination capacity relative to the control;
- Half a dose of the fungicide resulted in a 1.5% increase in the germination capacity relative to the control, but a dose of $1 \text{ L} \cdot \text{ha}^{-1}$ reduced this value by 0.3%.

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