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THE IMPACT OF APPLYING BIOLOGICAL TREATMENT ON THE INFECTION OF POTATO TUBERS BY THE FUNGUS *RHIZOCTONIA SOLANI* AND THE BACTERIUM *STREPTOMYCES SCABIEI*

Summary

The impact of applying Proradix WP (Pseudomonas spp. bacteria) and FZB24 WG (Bacillus subtilis bacteria) – biological treatments – on the infection of potato tubers by the fungus Rhizoctonia solani and the bacterium Streptomyces scabiei has been studied in the years 2011-2013. The experiment was conducted in a randomized split-block method, in 3 repetitions. Infection rate of tubers by Rhizoctonia solani, after applying the Proradix WP seed treatment, was significantly lower than in case of not treated ones. However, after applying the FZB24 WG seed treatment the infection rate was similar in both treated and not treated tubers. The infection rate of tubers by Streptomyces scabiei did not depend on tested biological treatments, the cultivar of the plant, or the weather conditions during the years of research. However, the weather conditions in 2012 had a significant impact on the occurrence of a higher percentage of tubers with symptoms of Rhizoctonia solani infection. Three potato cultivars did not differ in terms of infection by Rhizoctonia solani. In contrast, the percentage of tubers of Finezja cultivar infected by Streptomyces scabiei was significantly lower than in case of the Cyprian cultivar.
***Key words**: biological treatment, potato, health, R. solani, S. scabiei*

WPŁYW STOSOWANIA ZAPRAW BIOLOGICZNYCH NA PORAŻENIE BULW ZIEMNIAKA PRZEZ GRZYB *RHIZOCTONIA SOLANI* I BAKTERIĘ *STREPTOMYCES SCABIEI*

Streszczenie

W latach 2011-2013 badano wpływ dwóch zapraw biologicznych Proradix WP (szczepy bakterii Pseudomonas spp.) i FZB24 WG (szczepy bakterii Bacillus subtilis) na porażenie bulw trzech odmian ziemniaka przez grzyb Rhizoctonia solani i bakterię Streptomyces scabiei. Doświadczenie przeprowadzono metodą losowanych podbloków, w 3 powtórzeniach. Porażenie bulw przez Rhizoctonia solani po zastosowaniu zaprawy Proradix WP było istotnie niższe niż bulw niezaprawionych. Natomiast po zastosowaniu zaprawy FZB24 WG porażenie bulw przez tego patogena było podobne, jak bulw niezaprawionych. Porażenie bulw przez Streptomyces scabiei nie zależało od zastosowanych zapraw biologicznych, badanych odmian oraz warunków pogodowych w latach 2011-2013. Warunki pogodowe w 2012 roku wpłynęły istotnie na wyższy procent bulw z objawami Rhizoctonia solani. Trzy badane odmiany ziemniaka nie były zróżnicowane pod względem porażenia przez Rhizoctonia solani. Natomiast procent bulw odmiany Finezja porażonych przez Streptomyces scabiei był istotnie niższy niż odmiany Cyprian.
***Słowa kluczowe**: zaprawa biologiczna, ziemniak, zdrowie, R. solani, S. scabiei*

1. Introduction

Common scab and black scurf are serious potato diseases, which result in their lowered market, processing and seeding values. Black scurf (*Rhizoctonia solani* Kühn) symptoms include the rotting of sprouts, decay of stem bases, and presence of dark sclerotia on the surface of tubers. The infection of *R. solani* spreads best in low temperatures and high humidity of soil [1]. The common scab is caused by eubacteria *Streptomyces scabiei* (ex Thaxter) Lambert et Loria, *S. acidiscabies* Lambert et Loria (USA, Korea, Japan), *S. turgidiscabies* (Korea, Japan, Finland), *S. europaeiscabiei* (few locations in Europe, Korea), and *S. stelliscabiei* (France) [2, 3]. The species of the biggest significance in potato cultivation is *S. scabiei* [4]. The common scab usually occurs in slightly alkaline or neutral soils, especially during dry years [5]. The size of periderm scabs is the decisive factor of scab harmfulness. Tubers, which have stopped their growth and are covered with a well formed periderm are not susceptible to infection.

The sclerotia of *R. solani* and *S. scabiei* bacteria overwinter usually in soil and in the infected seed tubers [6]. These diseases can be limited by planting healthy seed tubers, crop rotation and chemical seed treatment of seed potatoes. Treating can result in equal rises, lowered plant density in the field and obtain healthy tubers yield. The success of using biological methods in orchards, greenhouses, and mushroom-compost houses protection has resulted in research of use of these methods in other cultivation fields [7]. Since 2014, the Directive No. 2009/128/WE [8], dictating the use of integrated methods, with priority to nonchemical and natural methods, also affects field crops. In recent years, the percentage of biological treatments usage has risen in the general pesticide market, however their use on large areas is still considered as expensive. It would seem, that in order to eliminate disease factors residing in soil, the use of beneficial, naturally occurring bacteria would be advised – through such environmental modification, which would result in stimulated growth and activity of mentioned bacteria [9]. The bacteria used in biological

protection can affect other organisms in an antagonistic way through antibiosis, competition for food and habitat, parasitism, and inducing systemic immunity in plants [10].

Extensive research is being conducted in the field of potato protection against *R. solani* and *S. scabiei*. It includes using the antagonistic bacteria *Bacillus* or *Pseudomonas* [11-20], conjunctive use of *Bacillus subtilis* bacteria and *Trichoderma virens* fungi [21, 22], as well as composts [23-25]. During the petri dish analyses, the inhibitive effects between fungi, actinomycetes or bacteria, isolated from the soil in which the tubers were cultivated, causing the common scab, have been studied [26, 27]. The effect of non-pathogenic strains of *Streptomyces* spp. on lowering of intensity of common scab is being studied [28, 29].

The aim of this research was to determine the effects of using two biological treatments: Proradix WP and FZB24 WG on health condition of three potato cultivars: Cyprian, Finezja and Flaming.

2. Materials and Methods

The research was conducted in the years 2011-2013 in the agricultural research station in Pawlowice, belonging to Wrocław University of Environmental and Life Sciences. The two-factor experiment has been conducted on brown earth (grade class IVb of "weak rye complex" complex, according to the soil grading system formed by the Polish Institute of Soil Science and Plant Cultivation), in a randomized split-plot method, in 3 repetitions. The experimental factors were: 1) Seed treatments: Control Group (no seed treatment), Proradix WP, and FZB24 WG; 2) Cultivars: Cyprian, Finezja and Flaming. The potatoes were treated with two biological preparations: Proradix WP – a microbiological agent designed against *R. solani* and *S. scabiei*, containing strains of *Pseudomonas* spp. bacteria; FZB24 WG – a fungicidal seed treatment containing a strain of *Bacillus subtilis*, naturally occurring in soil, effective against *Rhizoctonia* spp., *Fusarium* spp., and *Phytophthora* spp. (this seed treatment can be used in protection of potatoes, corn, vegetables and lawns [30]). The area of a plot equaled 12,5 m^2, with plant density of 60 tubers per plot. The studied cultivars seed tubers were treated directly before planting, with a dose of 75 ml of liquid seed treatment per 100 kg of potatoes. The potato yield was collected in the beginning of September of each research year.

In the years 2011-2013, the percentage of potato tubers infected by *R. solani* and *S. scabiei* has been calculated, as well as health condition of 3 potato cultivars (Cyprian, Finezja, and Flaming) has been studied. Before the harvest, 40 treated and not treated tubers of each cultivar have been analyzed and graded, in 3 repetitions. The degree of infection of tubers by *R. solani* and *S. scabiei* has been determined according to the gradual, inverted scale of COBORU (Polish Research Center for Cultivar Testing), modified by Moszczyńska. In this scale the values have following meanings: 1 – sclerotia or *R. solani* or scabs caused by *S. scabiei* occupied up to 1% of the tuber surface, 2 – from 1% to 10%, 3 – from 11% to 25%, 4 – from 26% to 50%, 5 – from 51% to 75%, 6 – more than 75% of the tuber surface.

The infection index has been calculated according to the following formula:

$$Wp = \Sigma (P \times W) \cdot n\text{-}1$$

Wp – infection index, P – the number of infected plants of specific infection degree, W – the value of infection, n – the number of graded plants.

The results of field research have been statistically analyzed with the method of variance analysis ($\alpha = 0{,}005$).

3. Results and Discussion

During the years of study, the percentage of potato tubers infected by *R. solani* was high. The biggest amount of tubers with sclerotia symptoms present on the surface was observed in 2012 (70% to 96%). In 2011 and 2013 the percentage of tubers infected by *R. solani* was lower and equaled (respectively) 15% to 64%, and 6% to 72%. Weather conditions in 2012 had a substantial effect on a lowered percentage of tubers with symptoms of infection by *R. solani* (Table 1.) According to some authors, the year of cultivation has the most significant influence on the rate and harmfulness of black scurf [31].

During the three years of study, the biggest amount of tubers with symptoms of black scurf was found amongst the tubers that were not treated. No significant statistic difference has been determined, considering the percentage of tubers infected by *R. solani* between the tubers that were treated with Proradix WP and FZB24 WG, and not treated tubers (Table 1, Figure 1).

Table 1. Percentage of potato tubers infected by *Rhizoctonia solani* depending on the type of seed treatment and the potato cultivar

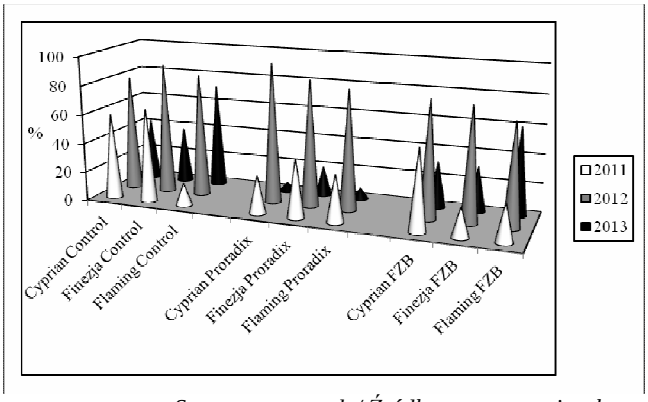
Tab. 1. Procent bulw porażonych przez Rhizoctonia solani w zależności od zastosowanych zapraw i odmian ziemniaka

Experiment variables		Years			Mean
Seed treatment	Cultivar	2011	2012	2013	
Control	Cyprian	58,33	79,17	42,50	60,00
	Finezja	64,17	90,00	38,33	64,17
	Flaming	15,00	84,17	71,67	56,94
Proradix WP	Cyprian	25,83	95,83	5,83	42,50
	Finezja	40,00	86,67	20,00	48,89
	Flaming	32,50	82,50	7,50	40,83
FZB24 WG	Cyprian	55,00	80,00	31,67	55,56
	Finezja	20,00	78,33	30,83	43,06
	Flaming	25,83	70,00	60,00	51,94
LSD p=0,05					r. n.
Means for variabiles					
Seed treatment	Control	45,83	84,44	50,83	60,37a
	Proradix WP	32,78	88,33	11,11	44,07a
	FZB24 WG	33,61	76,11	40,83	50,18a
LSD p=0,05					19,79
Cultivar	Cyprian	35,62	63,75	20,62	40
	Finezja	37,08	63,75	24,17	41,67
	Flaming	20,26	65,42	34,79	40,28
LSD p=0,05					r. n.
Years		31,11b	64,31a	26,53b	
LSD p=0,05					13,17

a, b – the differences of values followed by the same letters are not statistically significant

r. n. – differences not statistically significant

Source: own work / Źródło: opracowanie własne



Source: own work / Źródło: opracowanie własne

Fig. 1. The effect of using Proradix WP and FZB24 WG seed treatments on the percentage of potato tubers infected by *R. solani* (2011-2013 harvest)
Rys. 1. Wpływ zaprawy Proradix i FZB na procent bulw ziemniaków porażonych przez R. solani (zbiór 2011-2013)

The infection index of not treated and treated tubers ranged from 1,07 to 2,29; this means that the *R. solani* sclerotia occupied up to 10% of the tubers surface. The infection rate of potato tubers by *R. solani* after the usage of Proradix WP seed treatment was significantly lower when compared to the tubers that were not treated. However, the infection rate of tubers by this pathogen after the usage of FZB24 WG was similar to the not treated tubers (Table 2).

The research area concerning the biological protection of potatoes hasn't been studied enough by Polish researchers. Internationally, experiments pertaining to this matter include Petri dish analyses or greenhouse tests, field research is conducted in a lower than presently required amounts. Even though the spread of usage of biological treatments is quite limited, they can prove to be efficient against potato pathogens, especially in case of ecological cultivations. The Proradix WP is a biological treatment, based on *Pseudomonas* spp. bacteria. These bacteria secret siderophores, small, high-affinity iron chelating compounds, important biogenic components of many microorganisms. The *Pseudomonas* spp. improve the growth of the plants by influencing the change of composition of populations of microorganisms populating the rhizosphere, limiting the pathogens residing in soil. *Pseudomonas* strain Pf5 (producing pyrrolnitrin and pioluteorin) has been found to be an antagonist towards *R. solani* [20]. The results of a research conducted by others prove that *Pseudomonas* spp. reduced the growth of *R. solani*, caused the lyses' of sclerotia, lowered the frequency of occurrence of black scurf on tubers, and protected the tubers from infection by *R. solani* [11, 19]. The field research conducted in Germany, Italy, and Poland have shown that usage of Proradix WP seed treatment causes a drop in the occurrence of potato tubers infected by *R. solani* [32, 33]. A significant inhibition of black scurf has also been observed after the use of *Pseudomonas fluorescens* [12].

The *Bacillus* spp. strains or their metabolites can be considered as potential source of bio-pesticides and might be used in the control of phytopathogens, resulting in a decrease of fungicides usage. Supernatants obtained from the *Bacillus* bacteria cultures growing on brewery waste water and molasses, have shown an inhibitive activity towards such fungi as: *B. cinerea, R. solani, S. sclerotiorum, Phomopsis diachenii,* and *P. exiqua var. exiqua* [17]. The use of *Bacillus subtilis* and *R. solani* isolate of a low virulence significantly reduced the occurrence of the black scurf and common scab symptoms on potato tubers [21, 23].

Table 2. Index of the *Rhizoctonia solani* infection depending on the type of seed treatment and the potato cultivar
Tab. 2. Wskaźnik porażenia bulw przez Rhizoctonia solani w zależności od zastosowanych zapraw i odmian ziemniaka

Experiment variables		Years			Mean
Seed treatment	Cultivar	2011	2012	2013	
Control	Cyprian	1,58	1,87	1,48	1,64
	Finezja	1,64	1,96	1,43	1,67
	Flaming	1,15	1,84	1,74	1,57
Proradix WP	Cyprian	1,28	2,29	1,07	1,55
	Finezja	1,40	1,94	1,23	1,52
	Flaming	1,32	1,84	1,07	1,41
FZB24 WG	Cyprian	1,55	2,21	1,34	1,70
	Finezja	1,20	1,97	1,33	1,50
	Flaming	1,26	1,70	1,66	1,54
LSD p=0,05					r. n.
Means for variables					
Seed treatment	Control	1,46	1,89	1,55	1,63a
	Proradix WP	1,33	2,02	1,12	1,49b
	FZB24 WG	1,34	1,96	1,44	1,58ab
LSD p=0,05					0,12
Cultivar	Cyprian	1,36	1,84	1,23	1,48
	Finezja	1,37	1,72	1,26	1,45
	Flaming	1,20	1,66	1,37	1,41
LSD p=0,05					r. n.
Years		1,31b	1,74a	1,29b	
LSD p=0,05					0,13

a, b – the differences of values followed by the same letters are not statistically significant
r. n. – differences not statistically significant

Source: own work / Źródło: opracowanie własne

During the 3 years of study, the percentage of tubers infected by *S. scabiei* was low. In the years 2011-2012 the symptoms (scabs on the surface of tubers) were present in up to 10% of analyzed tubers, whereas in 2013 up to 20%. The biggest amount of infected tubers was present in case of tubers from the control group (2% to 12%), whereas the percentage of tubers with scabs on their surface after the seed treatment with Proradix WP and FZB24 WG ranged from 3% to 6% and from 2% to 9% respectively (Table 3, Figure 2). The usage of both studied biological treatments and the weather conditions during the 3 years of research have not affected the differentiating percentage of tubers with symptoms of the common scab (Table 3, Figure 2). In the years 2011-2013 the infection index of tubers from the control group and tubers that were treated with the studied preparations was low and ranged from 1,00 to 1,25; this means the scabs occupied up to 1% of the tubers surface. The degree of lesions caused by *S. scabiei* did not depend on the seed treatment used, the cultivar analyzed nor the weather conditions (Table 4).

However, the research conducted by others suggests that a significant reduction of the infection rate of potato tubers by *S. scabiei* could be achieved by using *Bacillus* sp. sunhua. The antibiotics produced by this bacterium (macrolactin A and iturin) effectively inhibited *S. scabiei* in a wide pH range and in high temperature [13]. *Bacillus amyloliquefaciens* and nonpathogenic strains of *Streptomyces* spp. also showed strong antagonism towards *S. scabiei* [16, 29]. Other researchers have proven a beneficial influence of *Bacillus subtilis*, *Bacillus thuringiensis*, a conjunctive use of *Streptomyces avermitilis* and *Trichoderma koningii*, and *Pseudomonas fluorescens* on a reduced infection rate of potato tubers by *S. scabiei* [14, 22].

Table 3. Percentage of potato tubers infected by *Streptomyces scabiei* depending on the type of seed treatment and the potato cultivar

Tab. 3. Procent bulw porażonych przez Streptomyces scabiei w zależności od zastosowanych zapraw i odmian ziemniaka

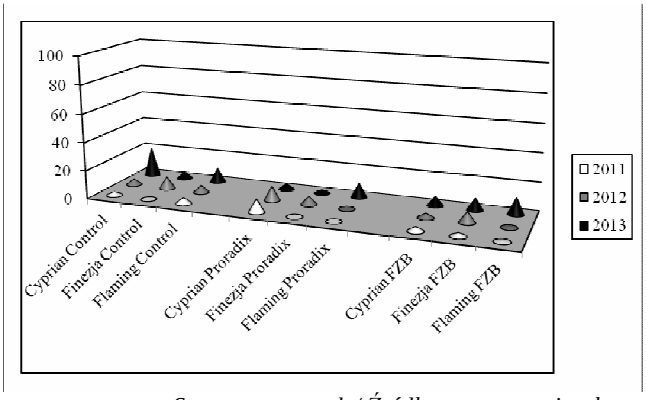
Experiment variables		Years			Mean
Seed treatment	Cultivar	2011	2012	2013	
Control	Cyprian	2,50	4,17	20,00	8,88
	Finezja	0,00	8,33	5,00	4,44
	Flaming	4,17	5,00	10,00	6,39
Proradix WP	Cyprian	9,17	10,00	4,17	7,78
	Finezja	0,00	5,83	2,50	2,78
	Flaming	0,00	0,00	10,00	3,33
FZB24 WG	Cyprian	3,33	3,33	6,67	4,44
	Finezja	2,50	7,50	8,33	6,11
	Flaming	0,83	0,83	11,67	4,44
LSD p=0,05					r. n.
Means for variables					
Seed treatment	Control	2,22	5,83	11,67	6,57
	Proradix WP	3,06	5,28	5,56	4,63
	FZB24 WG	2,22	3,89	8,89	5,00
LSD p=0,05					r. n.
Cultivar	Cyprian	13,54	10,21	8,33	10,69a
	Finezja	0,62	6,46	5,83	4,31b
	Flaming	7,08	1,46	10,42	6,32ab
LSD p=0,05					4,90
Years		7,08	6,04	8,19	
LSD p=0,05					r. n.

a, b – the differences of values followed by the same letters are not statistically significant

r. n. – differences not statistically significant

Source: own work / Źródło: opracowanie własne

The 3 studied potato cultivars (Cyprian, Finezja, and Flaming) were not differentiated in terms of percentage of tubers infected by *R. solani*, and the infection degree by this pathogen. However, in case of the common scab, the lowest percentage of tubers infected by *S. scabiei* was present in the Finezja cultivar and it was significantly lower than in the Cyprian cultivar (Table 3). According to other researchers, different potato cultivars were not significantly differentiated in terms of infection by *S. scabiei* and *R. solani* [31].



Source: own work / Źródło: opracowanie własne

Fig. 2. The effect of using Proradix WP and FZB24 WG seed treatments on the percentage of potato tubers infected by *S. scabiei* (2011-2013 harvest)

Rys. 2. Wpływ zaprawy Proradix i FZB na procent bulw ziemniaków porażonych przez S. scabiei (zbiór 2011-2013)

Table 4. Index of the *Streptomyces scabiei* infection depending on the type of seed treatment and the potato cultivar

Tab. 4. Wskaźnik porażenia bulw przez Streptomyces scabiei w zależności od zastosowanych zapraw i odmian ziemniaka

Experiment variables		Years			Mean
Seed treatment	Cultivar	2011	2012	2013	
Control	Cyprian	1,03	1,04	1,21	1,09
	Finezja	1,00	1,08	1,05	1,04
	Flaming	1,07	1,05	1,10	1,07
Proradix WP	Cyprian	1,13	1,10	1,04	1,09
	Finezja	1,00	1,25	1,02	1,09
	Flaming	1,00	1,00	1,10	1,03
FZB24 WG	Cyprian	1,04	1,03	1,06	1,04
	Finezja	1,03	1,07	1,08	1,06
	Flaming	1,01	1,01	1,12	1,04
LSD p=0,05					r. n.
Means for variables					
Seed treatment	Control	1,03	1,06	1,12	1,07a
	Proradix WP	1,04	1,12	1,06	1,07a
	FZB24 WG	1,03	1,04	1,09	1,05a
LSD p=0,05					0,06
Cultivar	Cyprian	1,14	1,10	1,08	1,11
	Finezja	1,01	1,11	1,06	1,06
	Flaming	1,10	1,01	1,10	1,07
LSD p=0,05					r. n.
Years		1,08	1,08	1,08	
LSD p=0,05					r. n.

a, b – the differences of values followed by the same letters are not statistically significant

r. n. – differences not statistically significant

Source: own work / Źródło: opracowanie własne

4. Conclusions

1. The infection rate of tubers by *R. solani* after the use of Proradix WP seed treatment was significantly lower than of tubers that were not treated.
2. In the future, the Proradix WP seed treatment might be used in biological protection against the black scurf, especially in ecological cultivations.
3. The infection rate of tubers by *R. solani* after the use of FZB24 WG seed treatment was comparable to tubers that were not treated.
4. In 2012 the weather conditions had a significant effect on a higher percentage of tubers showing symptoms of black scurf.
5. The beneficial effects of use of Proradix WP and FZB24 WG on the infection rate of tubers by *S. scabiei* were not observed, what may be the result of a very low infection rate of tubers by this pathogen during the 3 years of study.
6. The percentage of tubers of Finezja cultivar infected by *S. scabiei* was lower than of tubers of Cyprian cultivar.
7. The 3 studied potato cultivars (Cyprian, Finezja, and Flaming) were not differentiated in terms of infection by *R. solani* and *S. scabiei*.

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