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EFFECT OF THE CHEMICAL TREATMENT OF GOAT'S RUE SEEDS ON THEIR GERMINATION AND INFECTION WITH FUNGI

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Abstract. Increasing scope of the potential use of goat's rue (*Galega orientalis* Lam.) causes a growing interest in this plant also in research. Due to the structure of the seed coat of goat's rue, the seeds undergo scarification before sowing, which puts them at risk of pathogen infection. One of the more effective methods of disease prevention at the early developmental stages of plants is the application of chemical seed dressings, which may cause an increase in the proportion of dead seeds. Therefore, research on the response of goat's rue seeds to the generally applied seed dressings is advisable. An experiment was set up to establish the germination energy and germinability, as well as the proportion of goat's rue seeds (scarified or non-scarified) infected by fungi, treated with the following fungicides: Funaben T 480 FS, Raxil Gel 206 GF, Orius 02 WS, and Topsin M 500 SC. Goat's rue seeds were obtained from a long-term experiment (years 2008-2010 only). After harvest and seed threshing, under laboratory conditions, energy and germinability, as well as the number of infected by pathogens and non-germinating goat's rue seeds were determined. Observations were carried out after five and 14 days from the onset of germination. Analysis demonstrated that scarification increased seed germinability. Seed dressings did not affect the germination energy of non-scarified seeds, whereas scarified seeds displayed poorer germination energy in the first study season. Higher germinability was observed for treated versus untreated seeds, the differences between fungicides being significant only in 2008. It was also found that in the case of non-scarified seeds (after five days of germination), dressings did not diversify significantly the number of seeds infected by fungus diseases. After 14 days of germination, the number of seeds infected by fungi depended on the kind of dressing, season in which the seeds were collected, and the scarification process. Seeds, particularly the scarified ones, were best protected by dressings Funaben T 480 FS, Raxil Gel 206 GF, and Orius 02 WS.

Key words: fungicides, germinability, germination energy, scarification

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INTRODUCTION

Goat's rue (*Galega orientalis* Lam.) originates from the Caucasus region; it belongs to the family of Fabaceae [Komarov 1963, Ignaczak 2010, Kozłowski *et al*. 2012]. The plant easily adapts to the habitat conditions and performs very well when cultivated as a fodder crop, and as such is of high nutritional value [Varis 1986, Wojciechowska and Ignaczak 1992, Raig and Nômmsalu 2001, Sowiński and Szyszkowska 2002]. Fodder (green, ensilage, dried) made from goat's rue is rich in protein that contains all amino acids. Protein content varies, depending on the developmental stage of the plant, from 16% to 27% of dry matter [Kalembasa and Symanowicz 2003]. Ignaczak [2010] indicates that the species may also be used as protection for light soils periodically excluded from commodity production because goat's rue may comprise fallowing for 4-5 years and not require mowing during that period.

However, goat's rue has some unfavourable characteristics, including a high proportion of hard seeds, which can make up even 98% [Raig *et al*. 2001, Sowiński and Możdżeń 2007]. Seed hardness is an inbuilt characteristic associated with the structure of seed coat. The quality of seeds kept for sowing can be improved by mechanic or chemical scarification. Soaking of seeds in concentrated sulphuric acid is the easiest method of chemical scarification [Tworkowski *et al*. 1999, Sowiński and Możdżeń 2007]. This scarification method was applied in numerous studies and gave good effects, and scarification effectiveness depended on the plant species and the concentration and operation time of the chemical agent [Borkowska-Królik 1998, Mackay *et al*. 1995, Doliński *et al*. 2006].

All cultivated plants, including goat's rue, may be infected by pathogenic fungi and bacteria. The most frequent fungal diseases that infect young legume seedlings include root rot, grey mould, and *Alternaria* diseases. Seed treatment is one of the easiest and most economical methods of seed protection [Rane and Ruhl 2002, Siddiqui and Zaman 2004)]. However, fungicides may increase seed mortality and injury of seedlings [Moreno-Martinez *et al*. 1998].

Seed germinability is a widely accepted and frequently used indicator of sowing material quality [Contreras and Barros 2005]. Many factors affect this seed characteristic, including meteorological and agrotechnical conditions and seed treatment chemicals [Bieniaszewski and Fordowski 2001]. There is a paucity of literature on the effects of pre-sowing treatment of seeds on their germinability and infection by pathogens. Thus, the objective of the present work was to determine the effect of seed-treatment fungicides (Funaben T 480 FS, Raxil Gel 206 GF, Orius 02 WS, and Topsin M 500 SC) on the germination energy, germinability, and the number of goat's rue seeds infected by fungi.

MATERIAL AND METHODS

The data used in the present work were collected during three years (2008-2010) of a long-term experiment with goat's rue cultivar Gale established at the experimental unit of the University of Natural Sciences and Humanities in Siedlce (52°17' N; 22°28' E). The experiment was set up in 2005. Galega was cultivated for green matter in the first and second study years and for seeds in the subsequent three years. In each year, 170 single stems with fully mature pods were randomly sampled and dried to obtain airy dry matter. Seeds were collected on the following days: September 6th, 2008, September 4th, 2009, and September 12th, 2010.

Following harvest and threshing, the seeds were analysed in the laboratory to determine germination energy, germinability, and the number of pathogen-infected and non-germinating seeds depending on whether they had been scarified or not and according to the fungicide treatment applied (no treatment, Funaben T 480 FS, Raxil Gel 206 GF, Orius 02 WS, and Topsin M 500 SC). Active ingredients of the above chemicals are as follows: Funaben T 480 FS: carbendazim (148 $g \cdot dm^{-3}$) and thiram (332 $g \cdot dm^{-3}$), Raxil Gel 206 G: tebuconasole (6 $g \cdot dm^{-3}$) and thiram (200 $g \cdot dm^{-3}$), Orius 02 WS: tebuconasole (2%), and Topsin M 500 SC: tiophene (500 $g \cdot dm^{-3}$).

For the seeds to be scarified, they were soaked in concentrated H_2SO_4 for 17 minutes. Then, they were rinsed several times with running water in order to obtain pH = 7.0.

Seeds were manually coated with fungicides and immediately transferred into Petri dishes with a 90 mm diameter. One hundred seeds per dish were placed on three layers of damp cellulose filter paper and the contents of each dish were kept moist and at constant temperature throughout the whole experiment.

Germination energy and germinability were determined after five and 14 days of germination, respectively, by counting all the germinated seeds. Additionally, the number of seeds on which fungi started to grow was determined, thus indicating the development of fusariosis and damping-off after five and 14 days.

Statistical analysis of the proportion of seeds that germinated (after five and 14 days) was based on three-factor ANOVA (years, stratification type, and fungicide type). Before ANOVA, the results were Bliss-transformed, as they were not normally distributed [Trętowski and Wójcik 1991]. Mean separation was obtained by the Tukey's test at $P \leq 0.05$. As the number of seeds infected after five and 14 days had a non-parametric distribution (and it was not possible to select a suitable transformation), the Kruskal-Wallis test was used. All statistical analyses were performed using Statistica 9.0.

The temperature and precipitation during the growth seasons of 2008-2010 are presented in Table 1. The year 2010 was the warmest, as the temperature recorded in June and July was the highest in the whole three-year study period. The lowest precipitation at the beginning of the growth season (April-May) was recorded in 2009 and the highest in 2010. Every year, precipitation amount (recorded at the stage of setting pods) in June was higher than the minimum amount necessary to meet the water needs of goat's rue (24 mm). In August, the month that preceded seed collection, the highest monthly precipitation sums, as well as high monthly average temperatures were recorded in 2010. Those conditions caused seed collection in that year to take place the latest.

Table 1. Temperatures and precipitation during the growth seasons of 2008-2010
Tabela 1. Warunki termiczno-wilgotnościowe panujące w okresie wegetacji 2008-2010

Year Rok	Temperatures and precipitation – Warunki termiczno-wilgotnościowe						
	March marzec	April kwiecień	May maj	Jume czerwiec	July lipiec	August sierpień	March-August marzec – sierpień
Mean temperatures – Średnia temperatura, °C							
2008	3.1	9.1	12.7	17.4	18.4	18.5	13.2
2009	1.5	10.3	12.9	15.7	19.4	17.7	12.9
2010	2.4	8.9	14.0	17.4	21.6	19.8	14.0
Precipitation – Opady, mm							
2008	65.0	28.2	85.6	49.0	69.8	75.4	373.0
2009	40.4	8.10	68.9	145.2	26.4	80.9	369.9
2010	12.9	10.7	93.2	62.6	77.0	106.3	362.7

Source: authors' own compilation based on the data obtained from the Zawady Experimental Station
Źródło: opracowanie własne na podstawie danych uzyskanych z Rolniczej Stacji Doświadczalnej Zawady

RESULTS AND DISCUSSION

The germination energy of scarified and non-scarified *G. orientalis* seeds in relation to the fungicide treatment in 2008-2010 is presented in Table 2. The parameter was affected by the growth season. In 2010, the mean germination energy of seeds after five days was significantly higher (46.9%) than in the remaining study years due to well-distributed precipitation and high temperatures in June and July (Table 1). According to Slepetys [2001] and Sowiński and Możdżeń [2007], the weather pattern during the seed maturation stage determines the proportion of hard seeds, which, in turn, affects their germinability.

Table 2. Germination energy of goat's rue seeds depending on the fungicide and study year, %
Tabela 2. Zdolność kiełkowania nasion rutwicy wschodniej w zależności od zastosowanego fungicydu i roku badań, %

Fungicide Fungicyd	Year – Rok									Mean Średnia
	2008			2009			2010			
	N[1]	S	mean średnia	N	S	mean średnia	N	S	mean średnia	
No fungicide Bez fungicydu	13.5a*	79.3b*	46.4a	14.6a*	69.5a*	42.1a	18.3a*	72.3a*	45.3a	44.6ab
Funaben T	13.4a*	75.0a*	44.2a	14.4a*	70.0a*	42.2a	16.1a*	77.5b*	46.8ab	44.4a
Raxil Gel 206	14.5a*	74.7a*	44.6a	14.0a*	78.0b*	46.0b	17.0a*	78.6b*	47.8b	46.1b
Orius 02 WS	12.6a*	76.5ab*	44.6a	13.2a*	75.4b*	44.3ab	17.4a*	76.4ab*	46.9ab	45.3ab
Topsin M 500 SC	12.8a*	74.6a*	43.7a	13.5a*	78.1b*	45.8b	18.0a*	77.1b*	47.6ab	45.7b
Mean – Średnia	13.4*	76.0*	44.7A	13.9*	74.2*	44.1A	17.4*	76.4*	46.9B	45.22

[1] N – non-scarified seeds – nasiona nieskaryfikowane, S – scarified seeds – nasiona skaryfikowane
mean values in columns followed by the same small letters (a, b) do not differ significantly at $P \leq 0.05$ – średnie w kolumnach oznaczone takimi samymi małymi literami (a, b) nie różnią się istotnie przy $P \leq 0,05$
mean values in rows followed by the same capital letters (A, B) do not differ significantly at $P \leq 0.05$ – średnie w wierszach oznaczone takimi samymi dużymi literami (A, B) nie różnią się istotnie przy $P \leq 0,05$
* mean values in rows differ significantly at $P \leq 0.05$ – średnie w wierszach różnią się istotnie przy $P \leq 0,05$

Scarification by concentrated H_2SO_4 and the study years increased seed germination energy. Tworkowski *et al*. [1999] claims that H_2SO_4 scarification is effective because the process dissolves the cellular caps of seed coat palisade layer, which becomes permeable. As a result, the seeds start to lose water and exudates leak out. Also Sowiński and Możdżeń [2007] reported a favourable effect of concentrated H_2SO_4 scarification. In their study, non-scarified goat's rue seed germination energy ranged from 11 to 19.2% and, after scarification, it increased to 50.2-61.7%.

Seeds treated with Topsin M 500 SC and Raxil Gel 206 GF germinated better after five days of the experiment, reaching 46.1% and 45.7%, respectively. Moreover, there was a significant interaction of fungicides with the growth seasons and scarification. The fungicides did not affect the germination energy of non-scarified seeds. Compared with non-scarification, Topsin M 500 SC, Raxil Gel 206 GF, and Funaben T 480 FS reduced seed germination energy in 2008 by 4.7%, 4.6%, and 4.3%, respectively. A similar impact of thiram-based seed treatment was reported by Fordoński *et al.* [1994] and Faligowska *et al*. [2012], who studied pea, narrow-leafed lupin, and yellow lupin seeds. The authors noticed that seed germination energy dropped by 10%-25%. Scarified seed germination in 2009 was poorer (69.5%) compared with Topsin M 500 SC (78.1%), Raxil Gel 206 GF (78%), and Orius 02 WS (75.4%). The germination energy of non-scarified seeds in the last study year was similar to the one from 2009 (72.3%).

After 14 days of germination, the proportion of germinated seeds increased and was significantly higher in 2010 (55.79%) than in the remaining study years (Table 1). Scarification increased germinability in the successive study years. Over the three-year period, significantly lower germinability was recorded for non-treated seeds (44.5%) compared with seeds treated with Topsin M 500 SC (58.3%), Orius 02 WS (58.2%), Raxil Gel 206 GF (57.0%), and Funaben T 480 FS (55.9%). There was a significant interaction of the study years and scarification with fungicides. In all the study years, non-scarified and non-treated seeds had poorer germinability than fungicide-treated ones. The fungicides did not affect seed germinability. When scarified, seeds treated with Topsin M 50 SC had higher germinability than the ones treated with Funaben T and Raxil Gel 206 in 2008, whereas in the remaining study years, the germinability of fungicide-treated scarified seeds was higher than the one of non-treated seeds. No statistical differences were found between the fungicides (Table 3).

Statistical analysis demonstrated significant differences between the number of seeds infected by fungi depending on the fungicide, application of scarification, and the study years, as indicated by the values of the Kruskal-Wallis test (Table 4, Figs 1-3).

After five days of the experiment, numbers of non-scarified seeds on which fungi started to grow were the same for all the fungicides and the study years, whereas in the scarified seeds the extent of infection depended on the fungicide. The highest number of infected seeds was recorded for the control plots in all the study years. Topsin M 500 SC was not effective enough, as the proportion of seeds with symptoms of fusariosis and damping off development ranged from 4% to 11%. Cwalina-Ambroziak and Majchrzak [2000] also observed *Fusarium* fungi on goat's rue seeds. Fungi either did not grow or only slightly developed on seeds after the application of Funaben T 480 FS, Raxil Gel 206 GF, and Orius 02 WS in 2008 and 2010. Thus, seed treatment appears to be an important component of plant protection [Juszczak *et al*. 2001, Panasiewicz *et al.* 2008].

Table 3. Germinability of goat's rue seeds depending on the fungicide and study year
Tabela 3. Zdolność kiełkowania nasion rutwicy wschodniej w zależności od zastosowanego fungicydu i roku badań

Fungicide Fungicyd	Year – Rok									Mean Średnia
	2008			2009			2010			
	N[1]	S	mean średnia	N	S	mean średnia	N	S	mean średnia	
No fungicide Bez fungicydu	22.2a*	65.4a*	43.8a	23.2a*	62.3a*	42.75a	28.4a*	65.4a*	46.9a	44.5a
Funaben T	36.0b*	77.5b*	56.7cb	32.3b*	78.6b*	55.45b	33.6b*	77.6b*	55.6b	55.9b
Raxil Gel 206	31.1b*	78.1b*	54.6b	36.6b*	79.1b*	57.85b	37.2b*	80.1b*	58.65b	57.0cb
Orius 02 WS	33.2b*	81.2cb*	57.2c	37.0b*	80.2b*	58.6b	35.4b*	82.3b*	58.85b	58.2c
Topsin M 500 SC	35.2b*	83.2c*	59.2c	37.2b*	76.5b*	56.85b	36.5b*	81.4b*	58.95b	58.3c
Mean Średnia	31.54*	77.08*	54.31A	33.26*	75.34*	54.3A	34.22*	77.36*	55.79B	54.78

for explanations, see Table 2 – oznaczenia jak w tabeli 2

Table 4. Kruskal-Wallis test (H) values testing the effect of fungicides on the extent of infection of scarified and non-scarified seeds in 2008-2010
Tabela 4. Wartości testu Kruskala-Wallisa (H) testującego wpływ stosowanych fungicydów na porażenie nasion sakryfikowanych i niesakryfikowanych w latach 2008-2010

Observations made after Obserwacje zrobione po	2008		2009		2010	
Non-scarified seeds – Nasiona nieskaryfikowane						
5 days – 5 dniach	H = 5.69	p = 0.22	H = 8.44	p = 0.08	H = 6.71	p = 0.15
14 days – 14 dniach	H = 13.10	p = 0.01	H = 12.26	p = 0.01	H = 12.90	p = 0.01
Scarified seeds – Nasiona skaryfikowane						
5 days – 5 dniach	H = 12.01	p = 0.02	H = 12.12	p = 0.02	H = 11.84	p = 0.02
14 days – 14 dniach	H = 12.40	p = 0.02	H = 13.87	p = 0.008	H = 4.21	p = 0.37

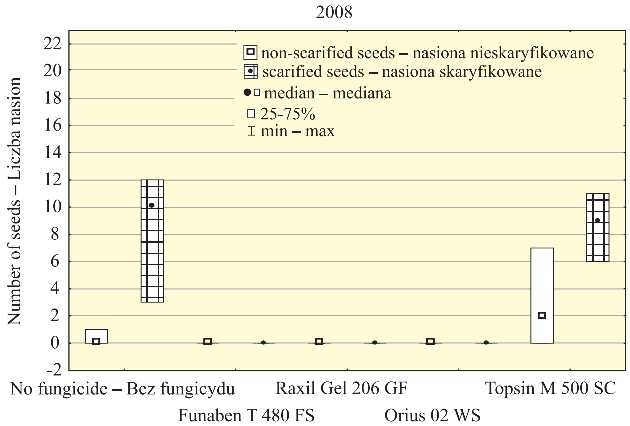

Fig. 1. Number of goat's rue seeds infected by fungi after five days depending on the applied fungicides in 2008
Rys. 1. Liczba nasion rutwicy wschodniej porażonych grzybami po pięciu dniach w zależności od zastosowanych środków grzybobójczych w 2008 roku

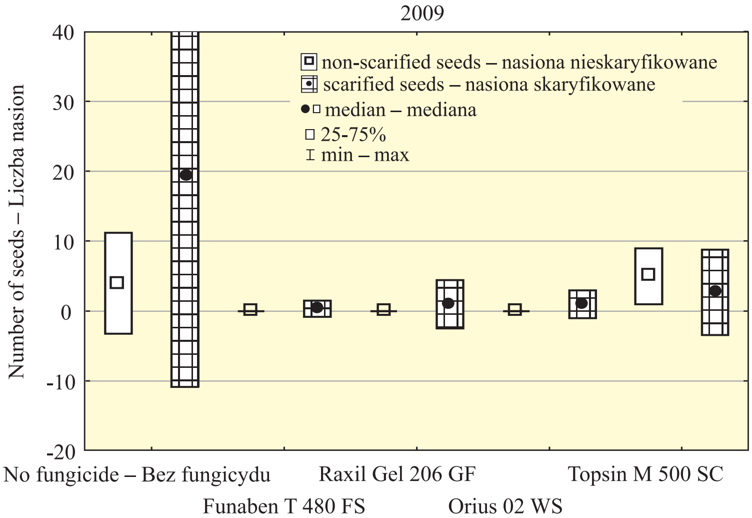

Fig. 2. Number of goat's rue seeds infected by fungi after five days depending on the applied fungicides in 2009

Rys. 2. Liczba nasion rutwicy wschodniej porażonych grzybami po pięciu dniach w zależności od zastosowanych środków grzybobójczych w 2009 roku

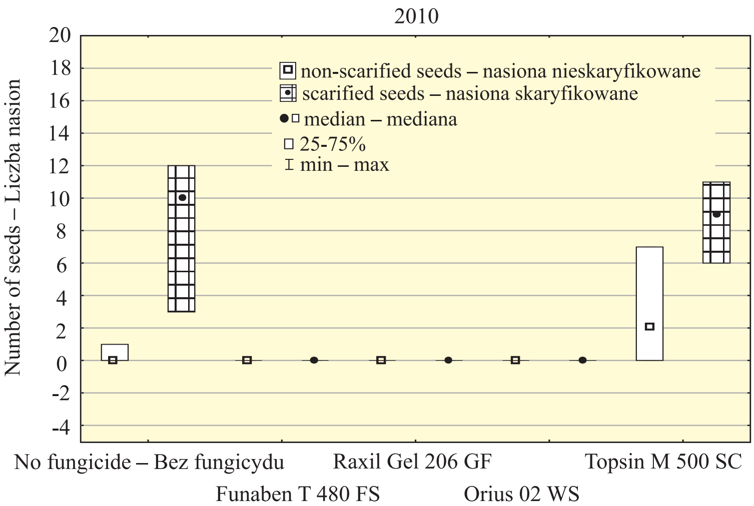

Fig. 3. Number of goat's rue infected by fungi after five days depending on the applied fungicides in 2010

Rys. 3. Liczba nasion rutwicy wschodniej porażonych grzybami po pięciu dniach w zależności od zastosowanych środków grzybobójczych w 2010 roku

After 14 days of germination, the control seeds were infected to the greatest extent. Funaben T, Raxil Gel 206, and Orius 02 WS provided the best protection of both scarified and non-scarified seeds collected in 2008 and 2009, and of non-scarified seeds in 2010. The extent of infection of fungicide-treated scarified seeds was insignificant and fluctuated around 10% (Figs 4-6).

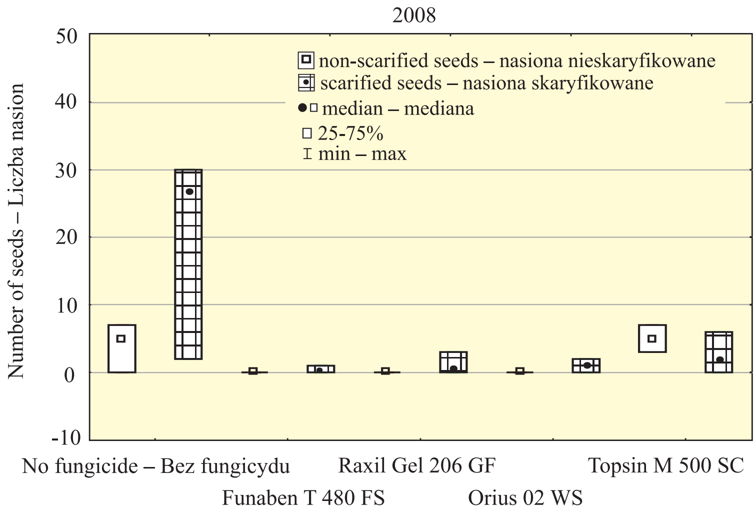

Fig. 4. Number of goat's rue seeds infected by fungi after 14 days depending on the applied fungicides in 2008

Rys. 4. Liczba nasion rutwicy wschodniej porażonych grzybami po 14 dniach w zależności od zastosowanych środków grzybobójczych w 2008 roku

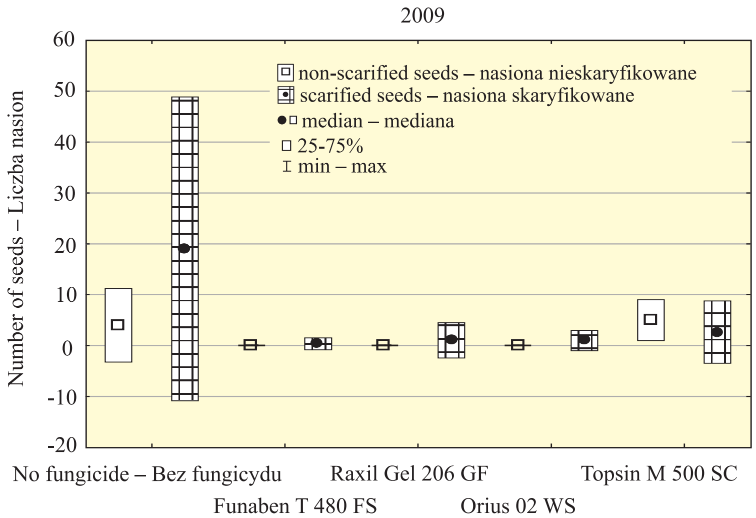

Fig. 5. Number of goat's rue seeds infected by fungi after 14 days depending on the applied fungicides in 2009

Rys. 5. Liczba nasion rutwicy wschodniej porażonych grzybami po 14 dniach w zależności od zastosowanych środków grzybobójczych w 2009 roku

The available literature provides information on the effectiveness of chemicals applied to treat the seeds of legume species, although according to Panasiewicz *et al.* [2008], there are not many works on the effect of seed-treatment chemicals on seed quality. Łacicowa [1981] found in her studies that systemic benzimidazole and carboxin-based fungicides are useful to combat diseases caused by fungi (*Fusarium* *solani* and *Fusarium phaseoli*). Seed germinability was the highest and the seedlings were the healthiest after the application of Benlate and Oxafun T. According to Narkiewicz-Jodko [1990], seed treatment, with Funaben T in particular, effectively reduces pathogenic and saprophytic microflora that develops on the seeds of legume species. Also Pięta and Pastucha [1993] point out that treating seeds with Funaben T and Oxafun T is necessary to protect soybeans.

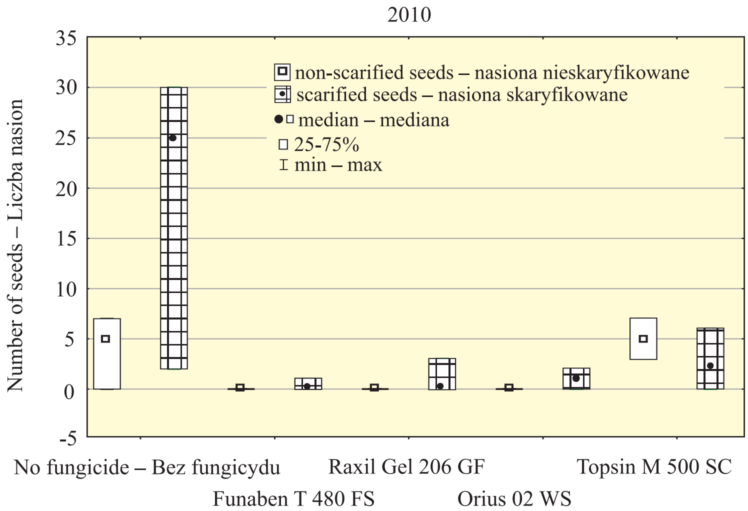

Fig. 6. Number of goat's rue seeds infected by fungi after 14 days depending on the applied fungicides in 2010

Rys. 6. Liczba nasion rutwicy wschodniej porażonych grzybami po 14 dniach w zależności od zastosowanych środków grzybobójczych w 2010 roku

The results of the study discussed above indicate that Funaben T 480 FS very effectively protects goat's rue seedlings against infection.

CONCLUSIONS

1. Scarified seeds of goat's rue were characterized by higher energy and germinability than non-scarified seeds.
2. Different dressings did not diversify the energy or germinability of non-scarified seeds.
3. Scarified seeds treated with Topsin M 500 S.C., Raxil Gel 206 GF, and Funaben T 480 FS, after five days of germination, had lower germination energy than non-scarified ones.
4. After 14 days of germination, scarified seeds treated with Topsin M 500 S.C. demonstrated higher germinability than seeds treated with Funaben T and Raxil Gel 206. The relation was observed only in 2008.
5. The number of non-scarified seeds infected by fungi was the same after five days of germination regardless of the applied treatment. After 14 days of germination, the best protection of both scarified and non-scarified seeds was given by treatments with Funaben T 480 FS, Raxil Gel 206 GF, and Orius 02 WS.

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WPŁYW CHEMICZNEGO ZAPRAWIANIA NASION RUTWICY WSCHODNIEJ NA ICH KIEŁKOWANIE ORAZ PORAŻENIE PRZEZ GRZYBY

Streszczenie. Rozszerzający się zakres potencjalnego wykorzystywania rutwicy wschodniej sprawia, że rośnie zainteresowanie tą rośliną również w badaniach naukowych. Budowa okrywy nasiennej nasion rutwicy powoduje, że przed wysianiem poddaje się je procesowi skaryfikacji, co z kolei naraża je na zainfekowanie przez patogeny. Jedną ze skutecznych metod zapobiegania chorobom w początkowych fazach rozwoju roślin jest stosowanie chemicznych zapraw nasiennych, które mogą wpływać na wzrost udziału nasion martwych. Celowe staje się więc prowadzenie badań dotyczących reakcji nasion rutwicy na powszechnie stosowane zaprawy nasienne. Doświadczenie polegało na zbadaniu energii, zdolności kiełkowania oraz udziału zainfekowanych przez grzyby nasion rutwicy wschodniej (nieskaryfikowanych i skaryfikowanych), zaprawianych fungicydami: Funaben T 480 FS, Raxil Gel 206 GF, Orius 02 WS and Topsin M 500 SC. Nasiona rutwicy wschodniej z lat 2008-2010 pochodziły z wieloletniego doświadczenia. Po zbiorze i omłóceniu nasion, w warunkach laboratoryjnych określono energię i zdolność kiełkowania oraz liczbę porażonych przez patogeny i niekiełkujących nasion rutwicy. Obserwacje przeprowadzono po 5 i 14 dniach od założenia doświadczenia. Analiza wykazała, że skaryfikacja wpłynęła na podwyższenie zdolności kiełkowania nasion. Zaprawy nasienne nie różnicowały energii kiełkowania nasion nieskaryfikowanych, natomiast u nasion skaryfikowanych obniżyły energię kiełkowania w pierwszym sezonie badań. Zdolność kiełkowania była wyższa u nasion zaprawianych niż niezaprawianych, a różnice pomiędzy zaprawami udowodniono tylko w 2008 roku. Stwierdzono ponadto, że w przypadku nasion nieskaryfikowanych (po 5 dniach od wysiania nasion) zaprawy nie różnicowały istotnie liczby nasion zainfekowanych przez choroby grzybowe. Po 14 dniach trwania doświadczenia liczba nasion, z których wyrastały grzyby, zależała od rodzaju zaprawy, sezonu, w którym zbierane były nasiona oraz od procesu skaryfikacji. Nasiona zwłaszcza skaryfikowane najlepiej chronione były przez zaprawy: Funaben T 480 FS, Raxil Gel 206 GF oraz Orius 02 WS.

Słowa kluczowe: energia kiełkowania, fungicydy, skaryfikacja, zdolność kiełkowania

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