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NOVEL METHODS OF TESTING BIOLOGICAL ACTIVITY OF SEED DRESSING FUNGICIDES AGAINST SNOW MOULD *MICRODOCHIUM NIVALE*

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Abstract: In the Institute of Plant Protection Branch in Sośnicowice an investigation was performed on establishing novel methods for testing biological activity of seed dressings against snow mould (*Microdochium nivale*). Seeds of winter wheat used for the experiments in artificial conditions were disinfected in sodium hypochlorite, subsequently infected with the pathogen, and treated with seed dressing fungicides (Baytan Universal 19.5 WS, Zaprawa Funaben T, Maxim 025 FS, Raxil Extra 515 FS and Vitavax 200 FS). In laboratory tests inoculation with conidial suspension was applied, and in climatic chamber experiments either conidial suspension or inoculum grown on sand-cornmeal medium was used. The field experiment with the same seed dressings was carried out on naturally infested plots, and the disease developed under snow cover lasting for 58 days. Two novel climatic chamber methods (Z and PK) proved to be suitable for testing biological activity of seed dressing fungicides against snow mould (*M. nivale*) as correlation coefficients with field results at P=0.05 were 0.9760 and 0.9453. Method Z was recognised as more suitable. Differences between seed dressings were statistically insignificant. The laboratory method was recognised as useful for controlling the pathogen (*M. nivale*), but its usefulness for controlling the disease (snow mould) may not be sufficient under a range of different winter conditions.

Key words: *Microdochium nivale*, snow mould, seed dressings, biological activity, methods of testing

I. INTRODUCTION

Microdochium nivale is well known as a causal agent of snow mould, the disease that occurs on winter cereals in the early spring at the time of melting of long-lasting snow cover. On the basis of extensive evidence it was accepted that snow mould appearing on the surface of winter cereal cultures as a profuse white-pinkish, sporulating mycelium, develops in those areas where snow cover has remained on the soil surface for over 100 days (Bojarczuk and Bojarczuk 1972; Łacicowa et al. 1987). According to Borecki (1987) early snow precipitation in the autumn, snow falling onto the unfrozen soil, and snow cover remaining in the field for over 80 days cause the most severe epidemics. The treshold for the occurrence of snow mould may be lower if other conditions favour its development. This was confirmed for conditions of Austria by Pichler (1952; 1957), who indicated that the disease may occur when snow remains in the field for 50 days or more. Long-lasting snow cover creates favourable conditions for the development of snow mould by preventing the access of daylight, increasing CO_2 concentration under the overlying snow, and maintaining high air humidity and temperature around 0°C (Drobnik and Kaczyński 1992). In the years 1991–1996 in Upper Silesia snow cover remained in the field for 91–110 days. This

favoured the development of snow mould, especially in the presence of a sufficient amount of inoculum. However, in the subsequent years snow precipitation started late and it remained in the field only for 58–73 days (Fig. 1). Because of shorter and snow-deficient winters of 1996–2000 and relatively short periods of snow cover *Microdochium nivale* more frequently caused seedling blights of winter cereals, although typical snow mould symptoms could also be seen. The pathogen also infects stem bases, leaves and ears, and the infection may occur at any plant growth stage. It has been shown that the infection of wheat plants during the vegetation period is mainly dependent on weather conditions and not on the stage of plant growth (Diehl and Fehrmann 1990).

Microdochium nivale is seedborne and can also survive in the soil and on plant debris being a source of primary infection of young plants in the autumn and early spring. Seed dressing with fungicides is the only method of reducing potential losses that may be very high in the years of snow mould epidemics. In consequence, new formulations to control *Microdochium nivale* are being developed and their efficacy estimated. In the Branch of Plant Protection Institute in Sośnicowice where biological activity of new preparations for disease control is tested with the purpose of authorization for the use in Poland, seed dressing efficacy against *Microdochium nivale* (snow mould) has recently been a subject of investigation. Generally accepted methods for testing biological activity of cereal seed dressings are not satisfactory for testing the activity against snow mould. Field testing is usually the most reliable, provided that weather conditions are suitable. As field testing is far too much time-consuming, faster and equally reliable methods designed for testing resistance of varieties and breeding lines of cereals to snow mould, or pathogenicity of the fungus are needed (Drobnik and Kaczyński 1992; Hömmö 1994; Koczowska et al. 1981; Łacicowa et al. 1978; Łacicowa et al. 1987; Madej and Porończuk 1995). Those are usually climatic chamber or glasshouse methods requiring controlled conditions. So far their use-

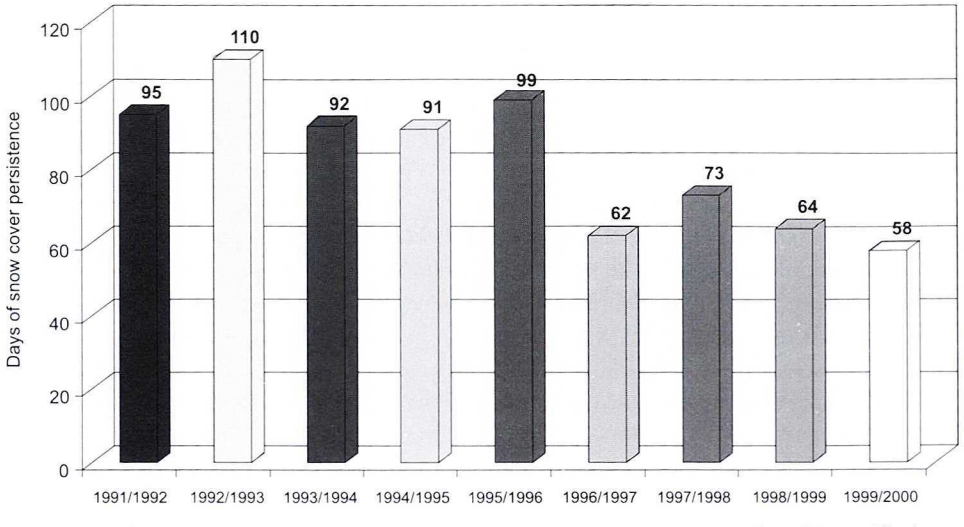

Fig. 1. Days of snow cover persistence from November to April in 1991–2000 in the region of Upper Silesia

fulness for testing biological activity of seed dressings against snow mould has not been recognised. The aim of this study was to design novel, faster methods producing results comparable to those obtained in field conditions.

II MATERIAL AND METHODS

Five preparations authorized for the use in Poland were chosen for winter wheat (cv. Roma and Kobra) seed dressing: Baytan Universal 19.5 WS, Zaprawa Funaben T, Maxim 025 FS, Raxil Extra 515 FS and Vitavax 200 FS and used in recommended doses. Experiments were performed in a climatic chamber, in laboratory conditions on Petri dishes, and in field conditions. As seeds are colonized by a range of various microorganisms including pathogenic species of fungi, a preliminary laboratory experiment was established to reduce their population. Seed disinfection was performed according to the following techniques:

I – 5% sodium chypochlorite, disinfection for 15 min,
II – 10% sodium chypochlorite, disinfection for 15 min,
III – autoclaving at 120°C for 15 min,
IV – rinsing with tap water,
V – non-sterilised.

One hundred seeds (25 seeds in 4 replications) for each experimental variant were plated on PDA Petri dishes acidified with lactic acid to pH ca 4.0–4.5 and incubated for at least 10 days at 20°C. After this period of time the colonization of seeds by fungi was determined. Estimation of seed germination from each experimental variant was performed on moist filter paper, according to Dorywalski et al. (1964). As the disinfection of seeds in 5% hypochlorite for 15 min gave the most satisfactory results (Tab. 1), this technique was used in subsequent experiments in climatic chamber tests where artificial infection with *Microdochium nivale* of previously disinfected kernels was performed. In each experimental variant 100 seeds were used (25 seeds in 4 replications). For estimation of biological activity of fungicides 2 climatic chamber methods were used: climatic chamber method PK and climatic chamber method Z.

Climatic chamber method PK

The inoculum of *Microdochium nivale* was prepared on sand-cornmeal (quartz sand and cornmeal in proportions 1 : 1). A highly pathogenic isolate of *Microdochium nivale* was grown on pelleted medium in Erlenmeyer flasks at room temperature for 4–6 weeks. Disinfected in 5% sodium hypochlorite seeds were treated with fungicides and planted in pots (ø 10 cm) filled with heat sterilized mineral soil, on the surface of which 25 g of sand-cornmeal inoculum per pot was evenly spread. After planting seeds were covered with 2 cm layer of soil. Pots were kept in climatic chamber for 4 weeks where controlled conditions favouring the development of the disease (temperature ca 5°C, 90% air humidity, photoperiod 12/12) were maintained (Porończuk and Porończuk 1987). At growth stage of seedlings 11–12, temperature was lowered down to 0–1°C, and pots were kept in the dark for subsequent 2 weeks. The infection of plants was estimated after 6 weeks from the date of planting, at GS

Table 1

Fungi colonising grain of winter wheat after use of different methods of disinfection
IPP Sośnicowice 2000

No	Method of disinfecting	% infected seeds with *Microdochium nivale*	% infected kernels by other microorganisms	
			Other *Fusarium* spp.	Remaining
1.	Control (without disinfecting)	12.00 b	10.00 a	91.00 c
2.	Rinsing with water	9.00 b	9.00 a	63.00 b
3.	5% sodium hypochlorite	1.00 a	3.00 a	14.00 a
4.	10% sodium hypochlorite	0.00 a	2.00 a	5.00 a
5.	Autoclaved	0.00 a	0.00 a	0.00 a
LSD P=0.01		7.92	10.44	20.56

11–13. Percentages of infected seedlings were recorded and % efficacy of seed dressings calculated according to Abbott formula.

Climatic chamber method Z

Wheat seeds disinfected in 5% sodium hypochlorite were sprayed with a suspension of conidia (1 mln conidia/1 ml). The suspension was prepared using 1 highly pathogenic isolate of the fungus and 25 ml of the suspension was applied to 100 g of seeds (Łacicowa et al. 1978). After 24 hr incubation inoculated seeds were treated with seed dressings and planted in pots (ø 10 cm) filled with sterile mineral medium (perlit) (Hömmö 1994). The subsequent incubation in the climatic chamber and estimation of efficacy of seed dressing fungicides were carried out as described for climatic chamber method PK.

Laboratory method

In the laboratory method autoclaved seeds were used. They were treated with *Microdochium nivale* spore suspension, as described for climatic chamber method Z, and dressed with fungicides. Afterwards treated seeds were plated on acidified PDA Petri dishes and placed for 10 days under NUV light, photoperiod 12/12, temperature 5–10°C, air humidity ca 90%. After 10 days colonization of kernels by *Microdochium nivale* was estimated.

Field experiment

The field experiment was performed in the season 1997/1998. Seeds of winter wheat cv. Roma dressed with fungicides were sown at the optimal agronomic date on 20 m^2 plots in replications, according to [EPPO Guideline 1997 PP 1/19 (3)] method (1997). The soil and seeds were naturally infested with *Microdochium nivale* after the previous season because of frequent occurrence of the pathogen on wheat (Głazek et al. 1998). The infection of wheat plants with *Microdochium nivale* was determined in the early spring of 1998, directly after snow cover has melted, using techniques described for climatic chamber tests. Field

estimation was confirmed in laboratory conditions, by making isolations of the pathogen on PDA Petri dishes.

Results of experiments were subjected to analysis of variance. Data obtained in climatic chamber and laboratory tests in vitro were compared with data of the field experiment by calculating correlation coefficients at P = 0.05.

III RESULTS AND DISCUSSION

Results of preliminary laboratory tests are shown in tables 1 and 2. In table 1 it can be seen that seeds were infested with *Microdochium nivale* and species of *Fusarium*. Per cent of non-disinfected seeds infested by those pathogens amounted to 12.0 and 10.0% respectively. Rising with water caused a decrease of seed colonization by ca 40%, and slightly lowered the number of seeds on which *Microdochium nivale* and *Fusarium* spp. were present. Seed disinfection for 15 min in 5 and 10% sodium hypochlorite was highly effective. The colonisation of kernels by fungi was 6- and 16 times lower in comparison to non-disinfected seeds. In the case of disinfection for 15 min in 5% sodium hypochlorite *Microdochium nivale* was present only 1% of kernels, while disinfection in 10% hypochlorite totally eliminated this pathogen. However, the use of 10% sodium hypochlorite for 15 min significantly lowered seed germination by 12% (Tab. 2). Consequently, it was not suitable for further climatic chamber experiments. On the contrary, disinfection in 5% hypochlorite did not affect seed germination and proved to be suitable.

Wheat seeds are usually colonized by a range of fungi and bacteria (Błaszkowski 1994; Jańczak and Pokacka 1995; Mathur and Cunfer 1993; Porończuk and Madej 1998). In the described here experiments the fungal flora colonizing wheat seeds (Tab. 1) mainly consisted of *Alternaria* spp. and *Epicoccum purpurescens*. These species usually occur in great number on winter seeds (Błaszkowski 1994; Jańczak and Pokacka 1995; Michail 1989). *Fusarium culmorum, F. avenaceum, Mucor* sp., *Penicillium* spp. *Trichoderma viride* and *Septoria* sp. were also isolated, mainly from non-disinfected seeds.

The next stage of experimental procedure was to supply a sufficient amount of inoculum of *Microdochium nivale* on the surface of disinfected seeds, which is necessary to incite infection (Bojarczuk and Bojarczuk 1972; Koczowska et al. 1981; Łacicowa et al.

Table 2

Germination capability of wheat seeds assessed 8 days after sowing in laboratory conditions
IPP Sośnicowice 2000

No	Method of disinfecting	% germinated seeds
1.	Control (without disinfecting)	98.00 b
2.	Seeds rinsed with water	98.50 b
3.	5% sodium hypochlorite	98.50 b
4.	10% sodium hypochlorite	87.00 a
5.	Autoclaved	–
LSD P=0.01		3.23

Table 3

Comparison of different methods of assessment of seed treatment efficacy on control efficacy *Microdochium nivale* in winter wheat

IPP Sośnicowice 2000

No	Seed treatment	Dose per 100 kg grain (product – H_2O)	Methods of assessment							
			Field method**		Chamber method PK***		Chamber method Z***		Laboratory method***	
			% of infected plants	% efficacy*	% of infected plants	% efficacy*	% of infected plants	% efficacy*	% of infected kernels	% efficacy*
1.	UNTREATED	–	7.38 b	–	79.97 b	–	88.17 b	–	100.00 b	–
2.	Baytan Universal 19,5 WS	200 g + 900 ml	0.00 a	100.00	19.25 a	75.93	10.00 a	88.66	2.00 a	98.00
3.	Zaprawa Funaben T	200 g + 800 ml	0.05 a	99.32	21.18 a	73.51	15.33 a	82.61	0.00 a	100.00
4.	Maxim 025 FS	200 ml + 400 ml	0.26 a	96.47	22.16 a	72.29	12.93 a	85.34	1.75 a	98.25
5.	Raxil Extra 515 FS	200 ml + 400 ml	0.04 a	99.46	8.53 a	89.33	9.80 a	88.89	3.75 a	96.25
6.	Vitavax 200 FS	300 ml + 300 ml	0.00 a	100.00	15.83 a	80.21	7.14 a	91.90	0.00 a	100.00
LSD			1.06		14.69		13.00		3.83	
Coefficient of correlation for results obtained in the field and artificial conditions					0.9453		0.9760		0.9738	

* efficacy according to Abbott's formula
** P=0,01
*** P=0,05

1978; Madej and Porończuk 1995). The use of sand-cornmeal cultures of the pathogen or conidial suspension proved satisfactory (Tab. 3). In untreated control of climatic chamber tests (methods PK and Z) 79.97 and 88.17 per cent of seedlings were infected. If seeds were treated with seed dressing fungicides, 8.53–22.16 and 7.14–15.33% of plants were infected by *Microdochium nivale,* respectively. Differences between seed dressings were insignificant. Mean efficacy of fungicides reached the values 78.25% for method PK and 87.48% for method Z. In laboratory method with autoclaved and inoculated with conidial suspension kernels, after dressing with fungicides and incubation as described above, *Microdochium nivale* was reisolated from 0.0 to 3.75% of kernels. Mean efficacy of seed dressing was 98.4%. In the field experiment percentage of infected by *Microdochium nivale* plants was not high. On control plots sown with treated seeds this percentage ranged from 0.0 to 0.26. Mean efficacy of fungicides was higher than obtained in artificial conditions and amounted to 99.05%. Differences between seed dressings were insignificant.

In spite of considerable differences in percentages of infected seedlings, correlation coefficients with field results were 0.9453 for chamber method PK and 97.60 for chamber method Z. Correlation coefficient with results of laboratory method was 0.9738 (Tab. 3).

Field experiments conducted under natural conditions provide useful data only when climatic conditions favour the occurrence of snow mould (Cichy and al. 1998). The important factor for development of epidemic is also availability of inoculum. Seeds used for the experiments were harvested in 1997, when the incidence of *Microdochium nivale* infection of wheat plants in the region of Upper Silesia was relatively high, as compared to previous years (Głazek et al. 1998). The presence of infected plant debris remaining in the soil may be another source of infection (Nakajima and Abe 1996). In the winter of 1997/1998 snow cover has remained sufficiently long for the occurrence of typical symptoms of snow mould, although its intensity was not very high. Symptoms of seedling blight described by Koczowska et al. (1981) were also observed.

As snow mould occurs only when snow cover remains sufficiently long on the surface of soil, designing other reliable methods for testing biological activity of seed dressings is of crucial importance. The application of climatic chamber methods that require simulated conditions similar to those occurring in the field seem to be the most suitable. They provide results in a shorter period of time as compared to field experiments (Madej and Porończuk 1995). This was confirmed in our study, and both climatic chamber methods PK and Z proved to be satisfactory. Method Z was superior to method PK (Tab. 1). Laboratory method is fast and easy to perform, but it should be pointed out that it controls the fungus *in vitro*, but not the disease. High correlation coefficient of laboratory results with the results of field method was obtained in 1998, when the intensity of snow mould was rather low. It is not known what correlation coefficient would have been if the disease had been severe. Thus it seems to be advisable to conduct additional experiments under a wider range of conditions to verify this method and its suitability for practical use.

IV. CONCLUSIONS

1. Two climatic chamber methods described in this paper are suitable for testing biological activity of seed dressing fungicides against snow mould caused by *Microdochium nivale*. Correlation coefficients for results obtained with the above methods and field experiment results were 0.9453 for PK method ad 0.9780 Z method. Method Z was superior to method PK.
2. The efficacy of 5 seed dressing fungicides (Baytan Universal 19.5 WS, Zaprawa Funaben T, Maxim 025 FS, Raxil Extra 515 FS and Vitavax 200 FS) was high, and differences between preparations were not significant.

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VI. POLISH SUMMARY

NOWE METODY OCENY BIOLOGICZNEJ SKUTECZNOŚCI DZIAŁANIA ZAPRAW NA PLEŚŃ ŚNIEGOWĄ

W Oddziale Instytutu Ochrony Roślin w Sośnicowicach są prowadzone badania w celu rejestracji nowych zapraw między innymi na pleśń śniegową wywoływaną przez *Microdochium nivale*. Z powodu coraz częściej występujących trudności oceny skuteczności działania zapraw w ochronie ozimin przed tą chorobą (z jej typowymi objawami) i koniecznością potwierdzania w kontrolowanych warunkach objawów porażenia roślin przez *M. nivale* będącego również sprawcą m.in. fuzaryjnej zgorzeli siewek prowadzone były badania nad nowymi metodami oceny aktywności biologicznej zapraw przeciw pleśni śniegowej. Oceniano skuteczność działania kilku zapraw z różnych grup che-

micznych i o różnym mechanizmie działania w warunkach naturalnych oraz w warunkach sztucznej infekcji w doświadczeniach komorowych (Metoda Z i metoda PK) i laboratoryjnych. Współczynniki korelacji (P=0,05) dla wyników uzyskanych w komorze klimatycznej (metody Z i PK) oraz w laboratorium z wynikami doświadczenia polowego, w którym wystąpiła pleśń śniegowa, były wysokie i wynosiły odpowiednio 0,9760, 0,9453 i 0,9738. Przydatność metody komorowej Z do oceny biologicznej skuteczności działania zapraw nasiennych przeciwko pleśni śniegowej oceniono wyżej niż przydatność metody PK. Stwierdzono, że chociaż metoda laboratoryjna jest łatwa i prosta, to służy raczej do oceny wpływu zapraw na sprawcę choroby (grzyb *M. nivale*), a nie na występowanie pleśni śniegowej.