

***Pyrenophora teres*, HOST-PATHOGEN INTERACTION IN BARLEY UNDER SOME SEED TREATMENT CONDITIONS**

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Abstract

The paper aimed to present alternative against the pathogen *Pyrenophora teres* (anamorph *Drechslera teres*) which parasitizes barley crop in western parts of Romania even beginning with late autumn period, observing the evolution and symptoms spreading of this fungus. Across the world, *Pyrenophora teres* causing the net blotch of barley is regarded as the major foliar disease in *Hordeum vulgare* L. Throughout its two form of *Pyrenophora teres*, however different from genetical point of view, *P. teres* f. sp. *maculata* and *P. teres* f. sp. *teres* causes economic losses by reducing yield and grain quality and poor emergence in area with a high biological pressure of the pathogen. The trial extends for two years (2019-2020, 2020-2021) in the same area of cropping (monoculture system) using same seed treatment list and following the seed-borne cereal fungi assessment protocol [(EPPO 1/19 (4))]. Trial setup consisted in complete randomized blocks, 6 treatments like fludioxinil, fluxapyroxad and mixture, every plot measuring 10 m² and observations performed on 10 plants/1 m in length sample. When treated with two active ingredients the result obtained exerts an efficacy up to 98%, maintained in both trial years compared with the untreated plots where the pathogen was well established.

Key words: *Pyrenophora teres*, net blotch, pathogen, barley, efficacy.

INTRODUCTION

Net form net blotch (NFNB) of barley (*Hordeum vulgare*) is caused by the fungal pathogen *Pyrenophora teres* f. *teres*.

Globally, NFNB results in regular yield losses of between 10 and 40% with the potential for complete losses in environmental settings favourable to the pathogen, namely, susceptible cultivars with high sustained humidity and the absence of fungicides (Mathre et al., 1997; Liu et al., 2011).

Reduced or no-till agricultural practices have most probably contributed to the increase in importance of both forms net and spot form net blotch (NFNB and SFNB) disease (Mathre, 1997; McLean et al., 2009; Shipton et al., 1973); however, the susceptibility of current cultivars and trends in environmental conditions cannot be ruled out as contributing factors to the increased importance of the disease.

Originally named *Helminthosporium teres* (Sacc.) in 1809, the fungus was renamed

P. teres Drechs. (anamorph *Drechslera teres* (Sacc.) Shoem.) in 1930 (Shoemaker, 1959).

P. teres was subsequently divided into the two forms *P. teres* f. *teres* and *P. teres* f. *maculata* by Smedegård-Petersen (1971) based on the lesion types. *P. teres* f. *teres* develops necrotic lesions with distinct striations, developing the net-like pattern for which it was named. *P. teres* f. *maculata* develops oval necrotic lesions with a chlorotic halo (Shipton et al., 1973; Smedegård-Petersen, 1971).

Both forms induce a combination of brown necrotic spots/lesions and general chlorosis in affected barley leaves. The brown necrotic spots/lesions are induced by proteinaceous toxins (Sarpeleh et al., 2008; Bach et al., 1979 quoted by Sarpeleh et al., 2008) while the chlorosis has been shown to be induced by low molecular weight compounds (LMWCs) isolated from culture filtrates of *P. teres* (Weiergang et al., 2002).

The pathogen can infect and cause disease on leaves, leaf sheaths, stems and kernels of barley plants. Infection of the kernel can transfer the

pathogen into a new field and can serve as primary inoculum (Liu et al., 2011). Crop rotation, avoiding barley monoculture and eliminating or reducing primary inoculum in the field are means preventing the pathogen's development (Liu et al., 2011; Vasilieva et al., 2022).

Besides avoiding monoculture and use of seed treatments a choice of biocontrol methods, against diseases and pests, can lead to a safer crop production (Röhner et al., 2004; Grozea et al., 2015; Virteiu et al., 2016)

MATERIALS AND METHODS

To characterize the interaction between pathogen *Pyrenophora teres* with the host plant we used inoculated barley seeds (GEVES - France) and coated with different active ingredients, drilled in the last decade of November in year 2019, respectively 2020 in western part of Romania.

We chose various active substances as seed treatments: fluxapyroxad (100 ml/100 kg), fludioxonil (1.5 l/t), sedaxan (2 l/t), mixture of prothioconazole and tebuconazole (0.5 l/t) and sedaxan mixed with fludioxonil in a rate of 2 l/t.

The trial set up was done following EPPO and CEB guidelines for seed borne cereal fungi and seed treatments, EPPO 1/19 (4) Seed borne cereal fungi; 1/135 (4) Phytotoxicity assessment; 1/181 (4) Conduct and reporting of efficacy evaluation of trials including good experimental practices, CEB M042 Seed treatment.

Placed as randomised complete block, the plot has 10 sqm (1 m wide and 10 m length) 8 rows each plot (Figures 1-3).



Figure 1. Plots drilling with inoculated seeds



Figure 2 Trial setup and randomization

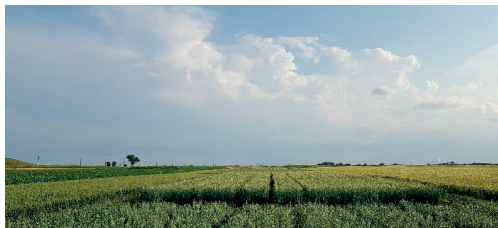


Figure 3. The research field in spring

We followed general phytotoxicity, crop vigour at early crop emergence and fully emerged (BBCH 12-14), speed of emergence and number of plant emerged, disease control (diseased plant/plot) assessing 10 samples/plot consisting in 30 plants per sample (Figures 4-6).

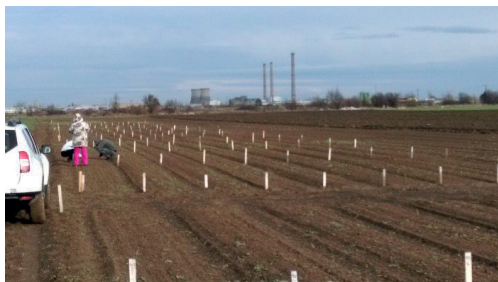


Figure 4. Performing assessment in late 2019



Figure 5. Assessing number of plants emerged and speed emergence



Figure 6. Assessment of diseased plant per plot

The data collected were statistically displayed throughout ANOVA and Student-Newman Keuls mean comparison test.

RESULTS AND DISCUSSIONS

Following the study protocol, general plant phytotoxicity and crop vigour at the first plots fully emerged were assessed. In tyhe plots where seeds treated with sedaxan mixed with fludioxonil and protoiconazole and tebuconazole as well the emergence and plant vigour was at highest level of 100%. The lowest emergence rate was recorded (in both experimental years 2019-2020) in untreated inoculated variant where the emergence noted was 70% as a mean value followed by untreated and not inoculated with 85%. The plant vigour proved to be in a strong relationship with the plant emergence rate, the most vigorous plants were those in the treatment 3 and 7, sedaxan/fludioxinil and protoiconazole/ tebuconazole respectively (Table 1, Figure 7).

Table 1. Plant vigour at BBCH 11

	Untreated		Untreated inoculated		Sedaxan + fludioxinil		Fluxapyroxad		Sedaxan		Fludioxinil		Propiconazole + tebuconazole	
	% general plants	% vigor plants	% general plants	% vigor plants	% general plants	% vigor plants	% general plants	% vigor plants	% general plants	% vigor plants	% general plants	% vigor plants	% general plants	% vigor plants
1	85	85	70	70	100	100	100	100	100	100	100	100	100	100
2	85	85	70	70	100	100	100	100	100	100	100	100	100	100
3	85	85	70	70	100	100	100	100	100	100	100	100	100	100
4	85	85	70	70	100	100	100	100	100	100	100	100	100	100
5	85	85	70	70	100	100	100	100	100	100	100	100	100	100
6	85	85	70	70	100	100	100	100	100	100	100	100	100	100
7	85	85	70	70	100	100	100	100	100	100	100	100	100	100
Average			87.5	70	100	100	95	95	95	95	95	95	100	100

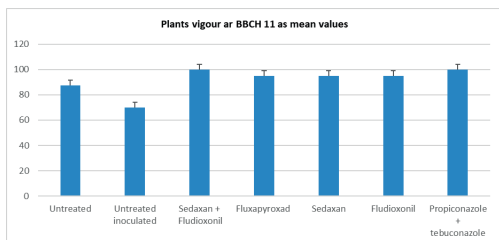


Figure 7. Plant vigour at fully emergence, BBCH 11

Following seeds treatments with the test items no general phytotoxicity was observed.

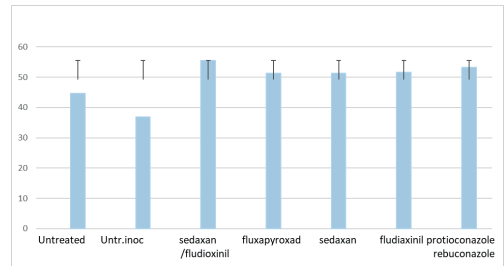


Figure 8. Plant emergence (plant/m²), 2019 St. dev. at $p < 0.05$

In respect of emerged plants/sqm, in year 2019, the differences are notable looking forward to yield potential being correlated to number of ears per m². It can be observed that in the plots where drilled untreated and untreated inoculated seeds, the number of plants was low, 36.8 plants per square meter in untreated inoculated plots respectively 44.7 plants/m² in untreated plots. The highest plant number was exerted in the plots where seeds are treated with sedaxan mixed with fludioxinil and protoiconazole plus tebuconazole, 55.5 plants/m² respectively 53.2 plants/m² (Figure 8).

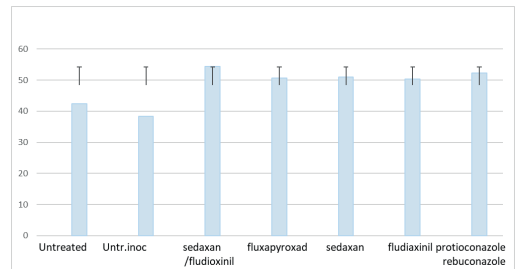


Figure 9. Plant emergence (plant/m²), 2020 St. dev. at $p < 0.05$

In experimental year 2020, the plants number per square meter did not change dramatically, dough a slightly increase was observed in the plots where untreated inoculated seed were drilled, in this case the emergence was 38.8 plants/m² (Figure 9). The highest emergence rate was recorded in variants where the seeds were coated with sedaxan plus fludioxinil at a rate of 2 l/t, with 54.3 plants/m². The variants where protoiconazole and tebuconazole mixture were applied the results were pretty

much in the same range as experimental year 2019, meaning 52.2 plants/m².

Table 2. Number of plants/m² in relation with untreated inoculated variant

	Untreated inoculated	Untreated	Fluxapyroxad	Fludioxonil	Sedaxan	Propiconazole + Tebuconazole	Sedaxan + Fludioxonil
Mean	36.81	44.71	55.52	51.24	51.29	51.57	53.24
St.dev	6.194	11.037	8.091	8.348	7.370	7.580	7.155
In % to UC	100	121	151	139	139	140	145

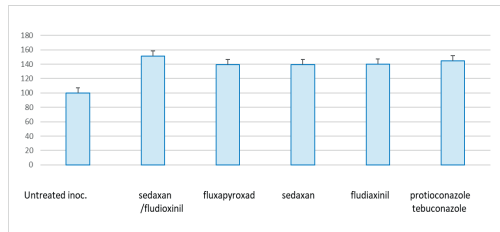


Figure 10. Plants per sqm in related to untreated inoculated

Regarding the disease control/diseased plants per variant, in year 2019 significant differences were found at $p < 0.05$ (Figure 11).

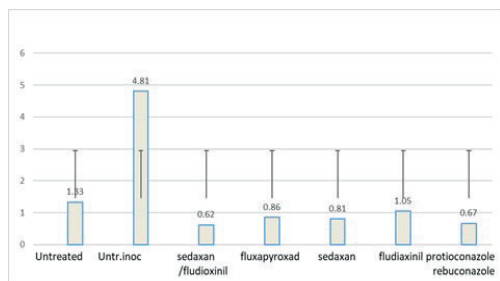


Figure 11. Diseased plants, 2019 significant difference at $p < 0.05$

The highest number of diseased plants, showing symptoms, was observed in variant where untreated inoculated seeds were drilled, namely 4.8 plants as mean value per variant. The lowest number of infected plants was achieved in variants where active ingredients sedaxan and fludioxinil was used to control seedborne pathogen *Pyrenophora teres* with a mean value of 0.62 plants infected. Despite the fact that untreated variants were drilled in conditions of natural occurring infection, the diseased plant rate lays in the range of 1.33 infected plants compared to 1.05 in the case of fludioxinil treatment (Figure 12)

In the next experimental year, 2020, however, like in previous year, significant differences were recorded between treatments.

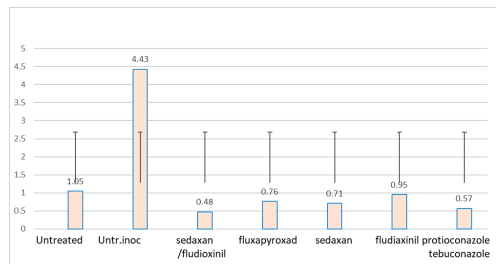


Figure 12. Diseased plants, 2020 significant difference at $p < 0.05$

Comparing untreated inoculated (4.43 diseased plants) variant with sedaxan and fludioxinil mixture one (0.48 diseased plants) and as Figure 10 shows, significant differences can be observed. The variants treated with protoconazole and tebuconazole. The untreated variant recorded an average of 1.05 diseased plants.

Treatments 4 and 5, namely seeds treated with fluxapyroxad at a rate of 100 ml/100 kg respectively treated with sedaxan at a rate of 2 l/t exerted no significant differences, the diseased plants number recorded was 0.76 and 0.71. whilst in experimental 2019 the infected plants as a mean value laid in the range of 0.81 in the case of sedaxan and 0.86 in the case of fluxapyroxad treatment.

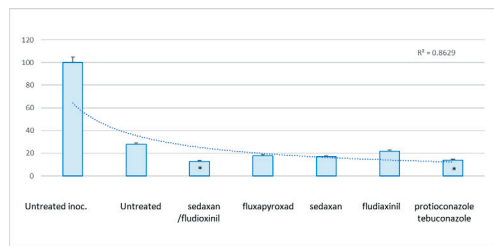


Figure 13. Effectiveness of seed treatments in year 2020, significant difference at $p < 0.05$

The efficacy achieved, in year 2020 (Figure 13), significant differences in the plots treated with sedaxan and fludioxinil mixture, where 13% of the exanimated plants compared with untreated inoculated variant, meaning an 87% of control. However, the variant where mixture of protoconazole plus tebuconazole was used the results were pretty much in line with the one obtained in above mentioned mixture (sedaxan and fludioxinil) with a efficacy up to 86% compared with untreated inoculated variant.

In respect of disease control, net blotch, can be approached through various methods, seed treatment playing an important role in disease epidemiology as an interrupting factor reducing the potential for secondary infections in the throughout vegetation period.

Although, the need of healthy food orientated to a healthy human diet and production of safe animal proteins (Manea et al., 2021), using the seed treatment with a right active substance which provide an extended period in control of soilborne pathogens can be linked with a sustainable, less costly and safer crop production at a farm level.

CONCLUSIONS

Pyrenophora teres causing net blotch is considered to be one of the most devastating diseases of cultivated barley (Weiland et al., 1999). Therefore, new technologies and active substances are developed including the ones used in seeds treatments.

The trial extends for two years (2019-2020, 2020-2021) in the same area of cropping (monoculture system) using same seed treatment list and following the seed-borne cereal fungi assessment protocol.

The lowest emergence rate was recorded (in both experimental years 2019-2020) in untreated inoculated variant where the emergence noted was 70% as a mean value followed by untreated and not inoculated with 85%, caused either by compromised germination through presence of seedborne pathogen in inoculated seeds or by biological soil reserve.

Nevertheless, in the variants treated with mixtures sedaxan and fludioxinil respectively prothioconazole and tebuconazole the emergence rate registered was 100%

Significant differences in respect of diseased plants per plots as a mean value was exerted by mixture sedaxan and fludioxinil with average of 0.48 diseased plants compared with untreated inoculated with an average of 4.43 – 4.81 diseased plants.

Fluxapyroxad used at a rate of 100 ml/100 kg and sedaxan used at a rate of 2 l/t exerted no significant differences, the diseased plants number recorded was 0.76 and 0.71.

In last years the farmers began to pay more

attention to new technologies implemented in whole food chain, especially entire technological process related to grain crops production where barley is included. So, there is a need to implement all the necessary steps to obtain productive and safer crops.

Other than usual procedures used in agriculture, such as soil preparation, crop rotation, and in vegetation treatments against pest and diseases, treatments, the use of treated seeds plays an key role in order to achieve profitable agricultural crops throughout decreasing of biological pressure of net blotch causing pathogen, *Pyrenophora teres*, as a soil seed borne pathogen, diminishing the second infection wave during the vegetation period of the crop through control within the crop and on secondary host as well.

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