sciendo

ASN, Vol. 9, No 3, Pages 84–92, 2022

Acta Scientifica Naturalis

Former Annual of Konstantin Preslavsky University of Shumen: Chemistry, Physics, Biology, Geography

Journal homepage: https://content.sciendo.com/view/journals/asn/asn-overview.xml

Duration of fungicide activity against wheat powdery mildew

Magdalena Koleva[1], Yordanka Stanoeva[2], Albena Ivanova[3], Plamen Chamurliyski[1]

[1] University of Shumen, Faculty of Natural Sciences, Department of Chemistry,
115 Universitetska Str., 9700 Shumen, Bulgaria
[2] Dobrudzha Agricultural Institute, 9500 General Toshevo, Bulgaria
[3] Technical University of Varna, 1 Studentska Str., 9010 Varna, Bulgaria

Abstract: *The most commonly used method to control wheat powdery mildew is by fungicides. The aim of the present study is to determine the duration of the fungicide activity of three fungicides: azoxystrobin; triadimenol; tebuconazole+spiroxamine+triadimenol. They were applied in three concentrations in phenophase 3-4 leaf of susceptible wheat variety. After treatment, each day one pot of the tested fungicide concentrations was inoculated with Blumeria graminis f. sp. tritici. When using azoxystrobin at concentrations of 0.2%, 0.4%, 0.8%, symptoms of powdery mildew appeared on plants inoculated with Blumeria graminis f.sp. tritici in the next day after treatment. When using triadimenol, first symptoms appeared in plants inoculated with the pathogen on the next day after applying a concentration 0.12%, on the second day after applying a concentration 0.25% and on the third day after treatment with a concentration 0.5%. With tebuconazole+spiroxamine+triadimenol, first postules appeared on plants inoculated with Blumeria graminis f. sp. tritici on the third day after treatment with a concentration 0.15%, on the 13th day after treatment with a concentration 0.3% and on the 14th day after treatment with a concentration 0.6%. This fungicide at a concentration of 0.6% (13 days) and 0.3% (12 days) has the longest duration of fungicide activity.*

Keywords: powdery mildew, *Blumeria graminis* f. sp. *tritici*, fungicides, wheat

Corresponding author: m.koleva@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0023
©2022 Magdalena Koleva et al., published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

Introduction

Wheat powdery mildew is a disease of economic importance for all wheat-growing areas, especially those with cool and temperate climates [1, 2]. In years with favorable climatic conditions, the disease can cause yield losses of 5 to 45% [3, 4], and when damaging the flag leaf yield losses can exceed 50% [5]. As a result of the disease, some quality indicators of the grain such as the amount of total protein and starch, alpha-amylase activity, decrease [6, 7, 8].

The disease is caused by the ascomycete, biotrophic, phytopathogenic fungus *Blumeria graminis* (DC) E. O. Speer f. sp. *tritici* Em. Marchal (Syn. *Erysiphe graminis* f. sp. *tritici* É.J. Marchal). In Bulgaria, the pathogen has a complete life cycle. The initial source of inoculum in the autumn are ascospores formed in cleistothecia at the end of the growing season of wheat [2]. Secondary source of inoculum are the conidiospores, which depending on the environmental conditions, can infect as early as February [9]. Temperatures of 10-22°C and humidity above 95% are favorable for disease development [9, 10]. The results of many studies have shown a high virulence diversity in the population of *B. graminis* f.sp. *tritici* in Bulgaria [2, 11, 12, 13, 14, 15, 16]. In 2018, Stanoeva [2] identified the distribution of 35 races of *B. g.* f.sp. *tritici* in different parts of the country, and race 7773 was reported for the first time.

The strategy to control wheat powdery mildew includes a complex of measures such as: use of resistant varieties, crop rotation, optimal sowing dates, optimal sowing density and fertilization rates, destruction of plant residues, and use of fungicides [17].

The use of resistant cultivars is the most efficient and cost-effective method to control wheat powdery mildew [9, 18]. The existence of sexual process and naturally occurring mutations in the population of *B. g.* f.sp. *tritici*, as well as the continuous cultivation of the same cultivars, lead to the emergence of new, more virulent pathotypes and races of the pathogen [19]. Therefore, breeding of wheat varieties resistant to *B. g.* f.sp. *tritici* is a very difficult task [11].

The most commonly used method to control wheat powdery mildew is by fungicides. A number of studies have shown the effectiveness of active substances from the groups of triazoles (flutriafol, triadimenol, triadimefon, diniconazole, tebuconazole, propiconazole, etc.), benzimidazoles, thiocarbamates, methoxyacrylates (azoxystrobin, pyramethostrobin, methominostrobin) in foliar treatment, as well as the active substance triadimenol in seed treatments [20, 21, 22]. Currently, the list of fungicides authorized for use in Bulgaria includes 57 products based on the individual or combined use of 27 active substances and most of them are registered for single and/or double application [23]. However, the duration of the fungicide activity remains unclear, and it is of primary importance to determine the interval between two treatments, as well as the number of treatments during the wheat critical phenophases: first-second node (BBCH 31-32) and flag leaf just visible - beginning of heading (BBCH 37-51).

Corresponding author: m.koleva@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0023
©2022 Magdalena Koleva et al., published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

The purpose of the present study is to determine the duration of the fungicide activity of some fungicides widely used to control wheat powdery mildew.

Materials and methods

The investigation was made under greenhouse conditions at Konstantin Preslavsky University of Shumen, College Dobrich. Wheat variety "Sadovska ranozreyka", susceptible to *B. g.* f.sp. *tritici*, was used. The plants were grown in 2L plastic pots in soil at 18°C/10°C (day/night) and photoperiod of 14 hours. After emergence, isolation chambers were placed on them [24].

Three fungicides authorized for use in the country in 2020 were used, and they will be noted only by their active substances: azoxystrobin (250 g/l), triadimenol (312 g/l) and tebuconazole (167 g/l) + spiroxamine (250 g/l) + triadimenol (43 g/l). Fungicides were applied in three doses: the registered one (Variant 1); two times lower (Variant 2) and two times higher (Variant 3), calculated on the basis of 20 l/da working solution and converted into percentages (Table 1).

Treatment with fungicides was carried out in the phenophase 3-4 leaf (BBCH 13-14) of the plants by spraying with a sprayer. For each of the variants tested, 20 pots were treated, in three replications, and 20 pots used as controls were sprayed with water.

Mass population of *B. g* f.sp. *tritici*, originated from North-Eastern Bulgaria, was used as inoculum. To obtain fresh spores, variety “Sadovska ranozreika was inoculated with the pathogen by spraying the spores on plants in the BBCH 11-12 [25].

Table 1. Fungicides applied for wheat powdery mildew control

Fungicide	**Registered dose, ml/da**	**Variant 1, %**	**Variant 2, %**	**Variant 3, %**
Azoxystrobin	80	0.4	0.2	0.8
Triadimenol	50	0.25	0.12	0.5
Tebuconazole+spiroxamine+triadimenol	60	0.3	0.15	0.6

Each day, starting the day after fungicide treatment, one pot of each fungicide test variant and one control were inoculated with *B. g.* f.sp. *tritici* in the manner described above. The plants were grown under the same conditions. Ten days after inoculation, disease intensity (DI) was estimated using the Cobb scale [26]. The area under the disease progress curve (AUDPC) was calculated:

$$\text{AUDPC}=\sum_{i=1}^{n-1}\left(\frac{x_i+x_{i+1}}{2}\right)\left(t_{i+1}-t_i\right),$$ where n – number of estimations; xi – disease intensity; ti (ti+1 - ti) – interval between two estimations.

Corresponding author: m.koleva@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0023
©2022 Magdalena Koleva et al., published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

Relative AUDPC (rAUDPC) was calculated as the ratio between the AUDPC of the respective sample and the AUDPC of the control [27].

The estimations continues until the DI of all tested variants of a given fungicide equals to that of the control.

Results and Discussion

When applying the fungicides in a concentration twice lower than the registered (Variant 2), the first postules of powdery mildew are observed on plants inoculated with *Blumeria graminis* f. sp. *tritici* the day after treatment with fungicides azoxystrobin and triadimenol (Table 2). For azoxystrobin rAUDPC = 1 and for triadimenol rAUDPC = 0.4. With tebuconazole + spiroxamine + triadimenol, the first postules on the inoculated plants were observed on the third day after treatment (rAUDPC=0.03).

The application of azoxystrobin at the registered concentration (Variant 1) led to the appearance of first symptoms of powdery mildew on the plants inoculated with the pathogen the day after treatment (rAUDPC = 0.20). With triadimenol in this variant, first symptoms appeared in plants inoculated two days after treatment (rAUDPC = 0.02), and with the fungicide based on three active substances, they appeared in plants inoculated 13 days after treatment.

Table 2. Relative area under disease progress curve of *Blumeria graminis* f. sp. *tritici* after application of three fungicides

Variants	F	Day												
		1	**2**	**3**	**4**	**6**	**8**	**10**	**11**	**13**	**14**	**15**	**16**	**17**
Variant 1	A	0.20**	0.30	0.80	1.00	-	-	-	-	-	-	-	-	-
	T	0.00	0.02	0.08	0.15	0.20	0.27	1.00	-	-	-	-	-	-
	TCT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.10	0.30	0.80	1.00
Variant 2	A	1.00	-	-	-	-	-	-	-	-	-	-	-	-
	T	0.40	0.40	0.50	0.67	0.83	1.00	-	-	-	-	-	-	-
	TCT	0.00	0.00	0.03	0.07	0.07	0.33	0.88	1.00	-	-	-	-	-
Variant 3	A	0.20	0.20	0.30	0.47	1.00	-	-	-	-	-	-	-	-
	T	0.00	0.00	0.02	0.15	0.20	0.27	1.00	-	-	-	-	-	-
	TCT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.20	0.70	1.00

* F – fungicide; A – azoxystrobin; T – triadimenol; TCT – tebuconazole + spiroxamine + triadimenol;

** Relative area under disease progress curve (rAUDPC)

At twice higher fungicide concentration (Variant 3), first symptoms of powdery mildew were observed on plants inoculated with *Blumeria graminis* f. sp. *tritici* on the next day after treatment with

Corresponding author: m.koleva@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0023
©2022 Magdalena Koleva et al., published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

azoxystrobin (rAUDPC = 0.20), on the third day after treatment with triadimenol (rAUDPC = 0.02) and on the 14th day after treatment with tebuconazole + spiroxamine + triadimenol (rAUDPC = 0.1).

The results of the present study show that when applying azoxystrobin at concentrations of 0.2%, 0.4% and 0.8%, symptoms of powdery mildew appeared on plants inoculated with *Blumeria graminis* f. sp. *tritici* the day after treatment. At a concentration of 0.4% (Variant 1) AUDPC reached control values in plants inoculated on the fourth day after treatment, at a concentration of 0.8% (Variant 3) on the sixth day, and at a concentration of 0.2% (Variant 2) the values were unity throughout the period (Table 2). The data show that at a concentration of 0.4% and 0.8%, the fungicide action is expressed not in a complete absence of symptoms, but in a lower disease intensity, which in turn means a smaller amount of inoculum for subsequent infections in real field conditions. The obtained results can be explained by the mode of action of the active substance, i.e. the lack of system action. Azoxystrobin is from the group of methoxyacrylates (strobilurins). It exhibits a translaminar action, blocks mitochondrial respiration, disrupts electronic energy transfer and ATP synthesis, as a result of which the germination of pathogen spores is inhibited [28, 29]. Currently, the list of authorized fungicides in Bulgaria includes 25 products that alone or in combination contain azoxystrobin [23]. Of these, 13 products are registered for control wheat powdery mildew, with nine containing only azoxystrobin, and four containing it in combination with another active substance.

When using triadimenol, first symptoms of powdery mildew appeared on plants inoculated with *Blumeria graminis* f. sp. *tritici* on the next day after application of concentration 0.12% (Variant 2), on the second day after application of concentration 0.25% (Variant 1) and on the third day after treatment with concentration 0.5% (Variant 3). This means that this fungicide in the registered concentration provides complete protection only one day after treatment, in twice higher concentration the period is two days, and in twice lower concentration does not provide complete protection to the pathogen. In lowest concentration, the AUDPC in the treated plants reached the control in plants inoculated eight days after application of the fungicide, and in the other two, ten days. It should be noted that the lower values of rAUDPC, at all three concentrations, provided a much smaller amount of initial inoculum for subsequent infections of *Blumeria graminis* f. sp. *tritici* under real field conditions. The product used in the study has not been authorized for use in the country since 03.2022 and there is currently no triadimenol-based product on the list of authorized fungicides [23]. Triadimenol is an active substance from the triazole group. Its mechanism of action is expressed by blocking the synthesis of ergosterol and ergosterol in the mycelium and spores of phytopathogens [29, 30]. For many years, commercial products on this base, alone or in combination, have been used extensively to control powdery mildews and rusts in many crops.

When using the third fungicide, a combination of active substances tebuconazole + spiroxamine + triadimenol, the first symptoms of powdery mildew appeared on plants inoculated with *Blumeria graminis* f. sp. *tritici* on the third day after treatment with a concentration of 0.15% (Variant 2), on the 13th day

Corresponding author: m.koleva@shu.bg Full Paper
DOI: 10.2478/asn-2022-0023
©2022 Magdalena Koleva et al., published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

after treatment with a concentration of 0.3% (Variant 1) and on the 14th day after treatment with a concentration of 0.6% (Variant 3). The AUDPC reached that of the control in plants inoculated with the pathogen 11 days after applying a concentration of 0.15% and 17 days after applying concentrations of 0.3% and 0.6%. Tebuconazole, like triadimenol, is an active substance also from the triazole group, with a similar mode of action. Based on it, alone or in combination, there are currently 40 commercial products registered, of which 16 can be used to control powdery mildew of wheat [23]. Spiroxamine is a systemically active substance from the group of morpholines. It inhibits sterol synthesis in fungal mycelial membranes, with a mechanism of action different from that of triazoles [29, 31]. Currently, spiroxamine is part of six products, four of which are registered against wheat powdery mildew, but not alone, and in combination with a triazole (most often tebuconazole) or strobilurin.

A comparative analysis between the tested three fungicides in three concentrations shows that the longest duration of fungicide activity, expressed in the complete absence of symptoms of powdery mildew, has the fungicide based on three active substances (tebuconazole + spiroxamine + triadimenol) at a concentration of 0.6% (13 days) and 0.3% (12 days). Differences in rAUDPC between these two concentrations were minimal. Two days is the duration of the fungicide activity of the same fungicide in concentration 0.15%, as well as with triadimenol in concentration 0.5%, and a day is fungicide activity of triadimenol in concentration 0.25%. The remaining tested concentrations of triadimenol and azoxystrobin did not show duration of the fungicide activity against *Blumeria graminis* f. sp. *tritici*. With the exception of azoxystrobin at a concentration of 0.4%, all tested fungicides and concentrations showed inhibition of disease development expressed by a lower AUDPC compared to the control for a different period of time (from three days with azoxystrobin 0.2% to 16 days with the combination of three active substances 0.3% and 0.6%).

Two of the fungicides used in the present investigation contain the active substance triadimenol, alone and in combination. The difference in the duration of the fungicidal action of the two products, in favor of the combined product, can be explained by the action of the active substances tebuconazole and spiroxamine alone or synergistically.

Conclusions

When using azoxystrobin at concentration of 0.2%, 0.4% and 0.8%, symptoms of powdery mildew appeared on wheat plants inoculated with *Blumeria graminis* f. sp. *tritici* in the day after treatment.

The first symptoms of the disease after treatment with triadimenol are observed on plants infected with the pathogen the next day after applying a concentration of 0.12%, on the second day after applying a concentration of 0.25% and on the third day after applying a concentration of 0.5%.

Corresponding author: m.koleva@shu.bg Full Paper
DOI: 10.2478/asn-2022-0023
©2022 Magdalena Koleva et al., published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

When using tebuconazole + spiroxamine + triadimenol, first symptoms of powdery mildew appeared in plants inoculated with *Blumeria graminis* f. sp. *tritici* on the third day after treatment with a concentration of 0.15%, on the 13th day after treatment with a concentration of 0.3% and on the 14th day after treatment with a concentration of 0.6%.

With all tested fungicides and concentrations, except for azoxystrobin at a concentration of 0.2%, suppression of disease development was observed, expressed by a lower AUDPC compared to that of the control for a different period of time (from three to 16 days).

Acknowledgement

This publication is realized with the financial support of Project No. RD-08-113/23.02.2022 of the Konstantin Preslavsky University of Shumen.

References

[1] Petrova, N.; Hsam, S.L.K.; Spetsov, P.; Zeller, F.,. Identification of powdery mildew and leaf rust resistance genes in common wheat [Triticum aestivum L. em. Thell.) cultivars grown in Bulgaria and Russia. *Plant Genetic Resources Newsletter*, **2000**, *122*, 32-35.

[2] Stanoeva, Y., Investigation of The Race Variability in Populations of Blumeria graminis tritici in Bulgaria during 2013-2015. In: Congress Book of 2nd International Balkan Agriculture Congress, 16-18 May, 2017, Turkey, **2018**, 44-49.

[3] Namuco, L.O.; Coffman, W.R.; Bergstro, G.C.; Sorrells, M.E., Virulence spectrum of the Erysispe graminis f.sp. tritici in New York. *Plant Disease*, **1987**, *71*, 539-541.

[4] Everts, K.L.; Leath, S.; Finney, P.L., Impact of powdery mildew and leaf rust in milling and baking quality of soft red winter wheat. *Plant Disease*, **2001**, *85*, 423-429.

[5] Alam, M.A.; Mandal, M.; Wang, C.; Ji, W., Chromosomal location and SSR markers of a powdery mildew resistance gene in common wheat line N0308. *African Journal of Microbiology Research*, **2013**, *7(6)*, 477-482.

[6] Gao, H. Y., Influence of powdery mildew on quality and its molecular mechanism in wheat (Triticum aestivum L.) Dissertation, Henan, China, **2012.**

[7] Gao, H. Y.; He, D. X.; Niu, J. S., The effect and molecular mechanism of powdery mildew on wheat grain prolamins. *The Journal of Agricultural Science*, **2014**, *152(2)*, 239−253.

[8] Feng, W.; Li, X.; Liu, W.D., Effects of powdery mildew infection on grain quality traits and yield of winter wheat. *Journal of Triticeae Crops*, **2014**, *34(12)*, 1706–1712.

[9] Stanoeva, Y.; Iliev, I., Dynamics of distribution of the cause agent of powdery mildew Blumeria graminis tritici on wheat during 2005-2009. *Turkish Journal of Agricultural and Natural Sciences*, **2014**, *Special Issue 2*, 1863-1869.

Corresponding author: m.koleva@shu.bg Full Paper
DOI: 10.2478/asn-2022-0023
©2022 Magdalena Koleva et al., published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

[10] Jones, D.G.; Clifford, B.C., *Cereal Diseases*: Their pathology and control. John Wiley and Sons, New York, **1983**.

[11] Iliev, I., Investigation on race diversity in populations of wheat powdery mildew pathogene in 1990-1991. *Plant Science*, **1996**, *33(2)*, 81-85 (Bg).

[12] Iliev, I., Investigation on the virulence Investigation on the virulence in the populations of the powdery mildew cause agent (Blumeria graminis f. sp. tritici) in Bulgaria during 1998-2000. *Plant science*, **2004**, *41*, 154-162 (Bg).

[13] Iliev, I., Virulence of the cause agent of powdery mildew in wheat during 2001 – 2003. *Plant science*, **2006**, *43*, 334-351 (Bg).

[14] Iliev, I., Virulence variability in the populations of the powdery mildew cause agent (Blumeria graminis tritici). *Plant science*, **2011**, *48*, 383-394 (Bg).

[15] Iliev, I., Race variability in the populations of the cause agent of powdery mildew in wheat during 2007 – 2009. *Field Crop Studies*, **2012**, *8(2)*, 227-238 (Bg).

[16] Stanoeva, Y., Dynamics of distribution of the powdery mildew on wheat in the Dobrudzha agricultural institute during 2016 – 2017. *Field Crop Studies*, **2019**, *7(4)*, 63-72, (Bg).

[17] Gao, H.; Niu, J.; Li, S., Impacts of Wheat Powdery Mildew on Grain Yield & Quality and Its Prevention and Control Methods. *American Journal of Agriculture and Forestry*, **2018**, *6(5)*, 141-147.

[18] Leath, S.; Heun, M., Identification of powdery mildew resistance genes in cultivars of soft red winter wheat. *Plant Disease*, **1990**, *74*, 747-752.

[19] Niewoehner, A.S.; Leath, S., Virulence of Blumeria graminis f. sp. tritici on wheat in the eastern United States. *Plant Disease*, **1998**, *82*, 64-68.

[20] Leath, S.; Bowen, K. L., Effects of powdery mildew, triadimenol seed treatment, and triadimefon foliar sprays on yield of winter wheat in North Carolina. *Phytopathology*, **1989**, *79(2)*, 152-155.

[21] Lipps, P. E.; Madden, L. V., Effect on fungicide application timing on control of powdery mildew and grain yield of winter wheat. *Plant Disease*, **1989**, *73(12)*, 991-994.

[22] Yang, L.; Zhou, Y. L.; Duan, X. Y., Sensitivity of Blumeria graminis f. sp. tritici isolates to triadimefon and fenpropidin in China in 2011. *Acta Phytopathologica Sinica*, **2013**, *43(4)*, 430–434.

[23] Bulgarian Food Safety Agency (BFSA), Fungicides, **2022** https://bfsa.bg/userfiles/files/RZ/Reg/2022/Fungicide%203.pd

[24] Reignault, Ph.; Cogan, A.; Muchembled, J.; Lounes-Hadj Sahraoui A.; Durand, R.; Sancholle, M., Trehalose induces resistance to powdery mildew in wheat. *New Phytologist*, **2001**, *149*, 519 – 529.

[25] Czembor, J. H.; Czembor, H. J., Powdery mildew resistance in selections from Moroccan barley landraces. *African Plant Protection*, **2001**, *7(1)*, 21–31.

Corresponding author: m.koleva@shu.bg Full Paper
DOI: 10.2478/asn-2022-0023
©2022 Magdalena Koleva et al., published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

[26] Peterson, R.F.; Campbella, A.B.; Hanon, A.E., A diagrammatic scale for estimating intensity on leaves on stems of cereals. *Canadian Journal of Research*, **1948**, *26*, 496-500.

[27] Li, Z. F.; Xia, X. C.; Zhou, X. C.; Niu, Y. C.; He, Z. H.; Zhang, Y. G.; Li, Q.; Wan, A. M.; Wang, D. S.; Chen, X. M.; Lu, Q. L.; Singh, R. P., Seedling and slow rusting resistance to stripe rust in chinese common wheats. *Plant Disese*, **2006**, *90*, 1302-1312.

[28] Joseph, Robert S.I., Metabolism of Azoxystrobin in Plants and Animals. In: *Pesticide Chemistry and Bioscience*, Woodhead Publishing, UK, **1999**.

[29] Nikolov, A., Phytopharmacy, Videnov &son, Sofia, (Bg), **2017**.

[30] Köller, W., Plant growth regulator activities of stereochemical isomers of triadimenol. *Physiologia Plantarum*, **1987**, *71(3)*, 309-315.

[31] Pesticide Properties DataBase, **2022**, https://sitem.herts.ac.uk/aeru/ppdb/en/

Corresponding author: m.koleva@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0023
©2022 Magdalena Koleva et al., published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License