Ph.D. thesis

Investigation of the quantity, translocation, degradation and effectiveness against *Fusarium* head blight of tebuconazole and prothioconazole after different spraying methods

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INTRODUCTION

Fusarium head blight (FHB) is a devastating disease of wheat (*Triticum aestivum* L.) that can give rise to serious losses in harvested grain. Besides reductions in yield and quality, harmful levels of mycotoxins can accumulate. One of the most important of these is deoxynivalenol (DON). The European Union have established a maximum DON content in unprocessed bread wheat of 1250 μg/g, in flour of 750 μg/g and in baby food of 200 μg/g. This criteria makes claims on farmers from the aspect of food safety of grains both for the national and the export markets.

Control of FHB can be achieved through various crop management practices that can reduce factors favoring for epidemic conditions. The most important variables which may reduce DON contamination are the previous crop, tillage, the resistance of wheat cultivar and the fungicide application. For lack of resistant wheat cultivars, in the case of environmental conditions favor for epidemic, the only effective way to protect the grain is the application of fungicides. Nevertheless fungicide application has not always good effectiveness.

Depending on the different fungicides, the biological attributes of cultivars and the environmental and methodological problems the effectiveness of chemical management moves in wide range. As concerns the fungicides used in FHB management triazole products were the most efficacious in reducing scab symptoms and DON content which was demonstrated in experimental circumstances and also in practice. Among triazoles tebuconazole which is in the market since 1988 and the relatively new prothioconazole, introduced in 2004, had the highest effectiveness. Besides the eminent efficacy, in some cases these two active ingredients (a.i.) were also inefficient in decreasing FHB symptoms or even caused higher DON content.

The contradictory results, found also on different type a.i., were explained by the sub-lethal doses of fungicides which act as a stress agent to the fungus, causing the induction of fungal defense processes and elevated toxin production. Namely fungicide effectiveness not only depends on the chemical type of a.i. but also on its concentration in the plant organs.

The fate of a.i. after spraying is usually strongly researched area from the aspects of environmental pollution. Moreover less is known about how these a.i. behave after landing on the plant surface within *in vitro* circumstances. Active ingredients may be grouped according to their uptake capacity by the plant and their mobility in and within plant organs. Tebuconazole and prothioconazole are known to systemic fungicides, which mean they can translocate within plants. Local a.i. movement may balance concentration differences within the treated organ, while long-distance translocation may be an advantage if a.i. move towards the infected organ, or disadvantage if the a.i. concentration decrease at the site of infection.

In the case of FHB of wheat, a.i. translocation – although poorly clarified – may be significant importance. Translocation from the flag leaves to the ears could increase a.i. content in the ears which have composite buildup, and low upper but extended lateral surface making difficult the uniform fungicide coverage. Traditional spraying methods are developed to the protection of leaves therefore nozzle types are set to spray vertically to protect as wide leaf area as possible. At the same time by this spraying method coverage of ears with their small upper surface is too low to have sufficient amount of a.i., causing lower protection level even if the timing and the spraying settlements are accomplished properly. In this case fungicide effectiveness may be much lower than expected.

Experiments were set up for enhance the fungicide coverage of wheat ears, and in this way increase the a.i. content and protection by targeting the spray directly to the heads. Nozzle types and adjustments were started to be used, with which streams could be angled backward and forward from 30° to 60° degree to target spray to the lateral surface of ears. These experiments were usually set up according to the spraying technology applied in the given countries, so there was no reliable data for Hungarian farmers.

By the research of the a.i., pathogen and host plant interaction and by the development of new highly effective spraying technologies not only the food safety can be secured but the environmental pollution can be also minimized.

In our research the highly efficient tebuconazole and prothioconazole are chosen to analyze the aspects of host plant–pathogen–fungicide interactions and to develop more effective spraying technology.

AIMS

- In greenhouse experiments long-distance translocation ability of prothioconazole and tebuconazole investigated after fungicide treatment at flowering.

- In small plot field trials the amount and distribution of a.i. between organs were investigated after hand spraying the plots. Furthermore the relationship between the amount of a.i. and effectiveness and the relationship with cultivars and environment were proposed to be clarified.

- In the spraying-technology development trials our aim was to investigate that how the different nozzle types and spraying directions can influence the amount and the abundance of a.i. within the plant organs and the effectiveness of chemical management.

- In all three different experiences degradation kinetics of prothioconazole and tebuconazole and the influence of environmental factors and cultivars were investigated during the most susceptible phenophase of of wheat.

MATERIALS AND METHODS

Plant material

Three wheat cultivars differing in *Fusarium* resistance were used in all tests: the GK Békés cultivar with awns, which is susceptible to FHB, and the awnless cultivars GK Kalász, which is also susceptible, and GK Fény, which is moderately resistant to FHB.

Fungicide

Throughout the entire experiment fungicide containing 125 g/l prothioconazole and 125 g/l tebuconazole was used for the treatments.

Greenhouse experiments

In the translocation test the fungicide mixture was applied in 3 different treatment groups in 3 repeats according to collection times. For the examination of basipetal movement, ears were treated, and for the examination of acropetal translocation, flag leaf blades were treated with fungicide. The movements of a.i.-s in the ears were tested by applying fungicide to the spikelets on the same side of the ear. Samples were collected at 2 h, and 2 and 4 days after treatment, except for the half ear treatment where samples collected at 2 h, and 2 and 4 days after treatment.

Small plot field trials

Field trials were carried out in Szeged in 2010 and 2011. Wheat cultivars were sown into 5.2 m^2 plots in 3 replicates per treatment, arranged in a randomized complete block design. The mix of 125 g ha^{-1} prothioconazole and 125 g ha^{-1} tebuconazole in 500 l ha^{-1} water was applied by hand sprayer. No additional adjuvant or surfactant was used. Untreated control plots were not sprayed by fungicide. The *Fusarium* inoculum was produced on Czapek-Dox liquid culture from the *Fusarium culmorum* isolates *F. c.* 12375 and *F. c.* 12551. Bunches of 15–20 ears were inoculated 2 days after fungicide spraying in both fungicide-treated and non-treated plots. All side of ears was covered with 15-20 ml inoculum. Bunches were covered with polyethylene bags for 48h to maintain moisture conditions. Disease symptoms were evaluated 12, 16 and 20 days after inoculation by the percentage of scabby spikelets for the whole group of ears. Harvested bunches were threshed and *Fusarium*-damaged kernels (FDK) were assessed visually as the percentage of scabby grains.

Spraying-technology development trials

Field trials were carried out in Kiszombor in 2010 and 2011. Winter wheat was shown at a rate of 550 seeds m^{-2} into corn debris to enhance inoculum source. Each plot of the varieties was divided into three parts according to the three different nozzle types. The plot shape adapted to the spraying equipment so they were 5.5 m wide and 70 m long in 2010 and 5.5 m wide and 40 m long in 2011. For control the same size of plots were used without fungicide treatment.

Spraying was made at mid-anthesis by a standard tractor-mounted field sprayer (Agromehanika AGS 600 E, 12 m boom). The spray volume was 250 l ha^{-1} driving speed was 8 km h^{-1}.The boom equipped with different nozzle types spaced 0.5 m apart from each other. Nozzle configurations were: 1) flat fan nozzle (XR TeeJet) used for spraying ears vertically; sideward spraying nozzles: 2) twin flat fan nozzle (Turbo TeeJet Duo) with medium drop size and 90 degree between fans; 3) wide angle flat fan nozzle (Turbo FloodJet) alternately forward and backward along the boom with very coarse droplets and about 120 degrees between fans. FHB was assessed 20 days after spraying.

For the measurement of ear coverage water sensitive papers (WSP) (TeeJet, Spraying Systems Co., Wheaton, USA) were fixed into a rod and stood among the wheat heads at the height of the ears. After spraying wsp-s were collected and coverage was calculated by pixel analysis software (GSA ImageAnalyser). For spray deposition assay tracer dye (0.2% ‘Green S’, Merck Chemical Ltd.) was mixed into the spray liquid. Coverage of randomly selected 20 ears assessed visually under UV light (365 nm wavelength) as percentage.

Sample collection

Field samples for a.i. quantification were collected 2 h and 2, 4 and 8 days after spraying. At each sampling time, 10 randomly selected ears with the stem containing the flag leaves were collected from each small plot of the 3 replicates of a given cultivar while in spraying-technology trials time 30 randomly selected plants were collected from the sub-plots of each nozzle. Collected plant material was stored at -20 °C until processing.

Analysis of a.i. and DON content

For a.i. quantification separated parts of the plants were-freeze dried (LYOVAC GT2, Leybold Heraeus GmbH, Köln, Germany) and weighed. Samples were cut into pieces or homogenized and extracted with 12.5 ml g^{-1} of an acetonitrile/water (8/2, v/v) mixture. Internal standard imazalil (Sigma-Aldrich Co., St. Louis, Missouri, USA) was added to the extract to correct for the variability of the chromatographic injection and the MS detection response. Recovery of the method for all analytes varied between 98 and 100%, depending on the plant organ and the a.i.. For DON quantification seed samples were homogenized and extracted with an acetonitrile/water (84/16, v/v) mixture, centrifuged (10 000 rpm) and filtered through Al_2O_3/activated charcoal.

Quantification of a.i. was performed on an HP 1090 liquid chromatograph connected to a Varian 500 MS ion trap mass spectrometer in positive ionization mode with an electrospray ionization (ESI) source. DON content was measured by high-performance liquid chromatography (HPLC) with DAD (diode-array) detection.

Statistical analysis

Statistical analyses were made with SPSS 19 software (IBM Corporation, New York, USA). Disease severity data were analyzed by three and four way analysis of variance (ANOVA) with *post hoc* Tukey HSD test, while a.i. content data were analyzed by repeated measures ANOVA (RMANOVA) with *post hoc* Bonferroni correction. Significant differences were set up at P≤0,05 level.

The degradation curves of a.i.-s were characterized by zero-order and first-order kinetics.

RESULTS AND DISCUSSION

Greenhouse experiments

In greenhouse experiments, after treating the flag leaf blade with the 1:1 mixture of prothioconazole and tebuconazole, there was no detectable prothioconazole acropetally in the ear. During the first 8 days after the treatments, no more than 3.13% prothioconazole-desthio and 0.5% tebuconazole were detected in the ear (to point of total a.i. content as reference). Prothioconazole-desthio was proved to be the most abundant metabolite of prothioconazole.

After ear treatments, 2.8% prothioconazole-desthio and 4.9% tebuconazole were detected basipetally in the flag leaf blade.

After the treatment of the spikelets on the half side of the ear, there was no prothioconazole translocation detected, while 1.1-6.4% prothioconazole-desthio and 2.2-19.8% tebuconazole were found in the flag leaf blades. Translocation between spikelets resulted in a measurement of 7.1-15.5% prothioconazole-desthio and 3.2-9.2% tebuconazole in the non-treated spikelets.

It can be concluded that no significant a.i. transclocation of compounds was come to pass. Neither acropetally nor basipetally could have been balance the possible concentration differences between the ear and flag leaf blade. Only moderate redistribution of a.i. was found between spikelets. The results suggest that prothioconazole and tebuconazole sholuld be sprayed as uniformly as possible into the ears to get the most proper protection.

Small plot field trials

In field experiments, when the fungicide was sprayed by hand on small plots, the concentration of a.i. (referred to dry weights) 2 hours after treatment was double in the flag leaf blade compared to the ears, nevertheless total a.i. content was higher in the ear at the same time.

In a very severe epidemic situation, the decrease of symptoms was even more than 40% in a susceptible variety, while at a less severe infection pressure, that is rather typical to wheat production, FHB decreased by more than 80%, the percentage of *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) content by more than 90%, when the initial content of prothioconazole-desthio was 1.70 (±0.41) μg in the ears and of tebuconazole 4.81 (±1.43) μg.

Spraying-technology development trials

In spraying-technology trials, following the usage of sideward-spraying Turbo TeeJet Duo, a.i. concentration in the ears was 1.4 times higher than after using the vertically-spraying XR TeeJet. Turbo TeeJet Duo is a twin type nozzle with medium sized droplets and 90° between forward and backward stream.

After spraying with Turbo FloodJet nozzles (large droplets, 120° between forward and backward steam) fungicide coverage and a.i. content changed by varieties and were remained unstable. The efficacy of this nozzle type was the same as Turbo TeeJet Duo's, when awned variety was sprayed. Awns can collect more droplets from the nearly horizontally discharged large droplets. After spraying with Turbo FloodJet, a.i. concentration in the ears was 1.2 times higher than after using the vertically-spraying XR TeeJet.

As comparing the the different spraying methods, for a.i. contents in the flag leaf blades after fungicide aplication, it can be concluded that the highest amount of a.i. was measured after using the vertically spraying XR TeeJet, that was in contrast with the results found in the ears. A 1.3 times increase occurred than with Turbo TeeJet Duo and 1.6 times increase than with Turbo FloodJet.

Total a.i. content in the overall wheat plants was nearly the same after spraying with XR TeeJet and Turbo TeeJet Duo, while it was much lower after using Turbo FloodJet. By means of changing the direction of spraying, the internal distribution of a.i. could also be changed, and enhanced in the ears.

In the spraying-technology trials what was carried out in 2010 under very severe epidemical conditions, FHB decreased on the average by 87%, FDK decreased by 81% and DON content was reduced by 75% after fungicide spraying. However, there was no significant difference found between the each nozzle types, as for disease decrease, that was very high with all three spraying methods.

Degradation of active ingredients

In the greenhouse experiments, the ratio of prothioconazole-desthio increased when the sampling time was lengthened as compared to prothioconazole, after treating the whole ear, the half side of the ear, and the flag leaf blade, respectively. Tebuconazole was proved to be the most stable compound, since its ratio was always higher, after the initial 1:1 ratio at the 2 hours samples, as compared to prothioconazole and its metabolite.

In the field experiments, the amount of prothioconazole was already measured under the detectable limit, 2 hours after spraying. Furthermore the amount of prothioconazole-desthio which was also a metabolite was much less than of tebuconazole, therefore we supposed the presence of other metabolites.

The speed of degradation of a.i. showed first order kinetics, but in some cases zero order kinetics was better fitted to the degradation curve, the latter probably caused by the relative short (8 days) measurement period. The minimum half-life of prothioconazol-deztio was 1.9 days that of tebuconazole was 5.3 days in the ears whereas this parameter was 1.7 days for prothioconazole-desthio, and 4.8 days for tebuconazole in the flag leaf baldes.

Prothioconazole-desthio content decreased faster than tebuconazole both in the ears and flag leaf blades. Both compound degraded faster in the flag leaf blade than in the ear. Comparing the three cultivars, the degradation of a.i. was the slowest in the GK Fény variety in both years and plant organs. These results reflect for quite new cultivar effect that may alter the efficacy of fungicides used.

CONCLUSION

Based on these results it can be summarised, that prothioconazole, its main metabolite prothioconazole-desthio and tebuconazole do not translocate between ear and flag leaf blade of wheat, after spraying at anthesis in a manner, that could balance the concentration differences among these plant organs. Accordingly, fungicide spray should be directed to the site of protection, namely to the ears, in order to get sufficient control. The tested fungicide decreased FHB symptoms with very good efficacy, but resistance of varieties had also significant effect. In the practice of FHB management, sideward-spraying nozzles with 90° between forward and backward stream, spraying with medium droplets, will enhance the fungicide coverage of ears as well as their active ingredient content.

ORIGINAL PUBLICATIONS DIRECTLY RELATED TO THE THESIS

Lehoczki-Krsjak Sz., Varga M., Szabó-Hevér Á., Mesterházy Á. Translocation and degradation of tebuconazole and prothioconazole in wheat following fungicide treatment at flowering *Pest Management Science*, DOI 10.1002/ps.3486 (In press)
IF: 2.251

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Lehoczki-Krsjak Sz., Szabó-Hevér Á., Tóth B., Kótai Cs., Bartók T., Varga M, Farády L. and Mesterházy Á. (2010) .Prevention of Fusarium mycotoxin contamination by breeding and fungicide application in wheat *Food Additives and Conaminants A*; Volume 27, Issue 5 May 2010. p 616 – 628
IF: 2,230

OTHER PUBLICATIONS

Szabó-Hevér Á., Lehoczki-Krsjak S., Tóth B., Purnhauser L., Buerstmayr H., Steiner B., Mesterházy Á. Identification and validation of fusarium head blight and *Fusarium*-damaged kernel QTL in a Frontana/Remus DH mapping population *Canadian Journal of Plant Pathology*, 2012. 34(2) p 224-238
IF: 0,884

Cumulative impact factor: 5,365