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Resistance behaviour of *Septoria tritici* to some fungicides in the territory of the Czech Republic

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Summary

Reduction of colony diameter using two DMI-fungicides and one strobilurine fungicide was evaluated in the *Septoria tritici* population. In an *in vitro* assay, 57 strains of *S. tritici* originating from different parts of the Czech Republic were grown on agar plates containing different concentrations of fungicides. The mean EC_{50} values of strains were analysed for flusilazole (Capitain), metconazole (Caramba) and one strobilurine fungicide: azoxystrobin (Amistar).

ED 50 values for metconazole were low. There were highly significant correlation in reaction of pathogen strains to metconazole and flusilazole. Some isolates showed significant higher level of resistance to azoxystrobin.

The data will be used in a continuing survey of resistance development in the *S. tritici* population originating from the territory of the Czech Republic.

Key words: resistance to fungicides, *Septoria tritici*, ED 50, flusilazole, metconazole; azoxy strobin

INTRODUCTION

Septoria tritici blotch caused by *Septoria tritici*, perfect state *Mycosphaerella graminicola* started to be a serious problem of wheat growing in the Czech Republic during the last two years. Disease epidemies developed early in the spring in both years 2002 and 2003 and damaged winter wheat stands in the whole area of the Czech Republic. These infection was important source for disease development in later growing stages.

Winter cereals are protected against fungal diseases by frequent spraying with DMI (inhibitors of C-14 demethylation of lanosterol or 24- methylendihydrolanosterol) fungicides. DMIs clearly belong to the group of site-specific fungicides, which are in general more prone to resistance than conventional multisite inhibitors (Dekker, 1985).

The strobilurins started to be an important class of agricultural fungicides during last decade. The huge impact of the strobilurin fungicides on agriculture is well reflected by the current status of azoxystrobin, which is now registered for use on 84 different crops in 72 countries (Bartlett et al., 2002).

In contrast to what was expected at the beginning of 2003 the spread of resistance of some cereal fungal pathogens was quite fast. In the monitoring programmes resistance was detected in all major wheat growing countries in Western Europe. Generally in-season monitoring data of 2003 show increased resistance levels compared to pre-season samplings, as well as further geographical distribution.

The aim of this work was to assessed possible development of resistance to fungicides from the both higher mentioned chemical groups. Received collection of *Septoria tritici* strains was subjected to the assessment of in vitro sensitivity.

MATERIALS AND METHODS

The methodological approaches are derived from the FRAC Methods for Monitoring Fungicide Resistance (1991).

Sample collection

Wheat plants with typical septoria leaf blotch symptoms were collected during early spring in the territory of the Czech Republic. There were collected 900 samples of winter wheat plants in 2002. One sample consisted of 100 plants from one field.

Leaves with *S. tritici* symptoms were surface sterilized in a 1% sodium hypochlorite solution for 3 min, rinsed in sterile water and dried between two sheets of sterile filter paper. Then the segments were cut into small pieces (2-3 mm). Finally, the segments were placed on potato-dextrose agar medium in Petri dishes.

14 day incubation in the dark at 21°C was followed by 4-days exposure to continuous UV light at 12°C. Three to four isolates were recovered from each location. After species identification, single spores of *S. tritici* were transferred to PDA and incubated for 7 days in the dark at 21°C as single spore cultures for the following tests. 52 isolates of *Septoria tritici* were used for this experiment.

Description of the sensitivity assay

Petri dishes containing PDA-agar with or without fungicides were prepared. The two DMI-fungicides used were metconazole and flusilazole, azoxystrobin was use as strobilurine fungicide. The following rates were tested: 0.0, 0.1, 0.25, 0.5, 1.0, 2.0, 5.0 and 10.0 $g \cdot ml^{-1}$ of fungicides. The test included three replications of each concentration/isolate/fungicide combination as well as three replications of the fungicide free controls.

The colony diameter was measured after incubation for 15 days in the dark at 21°C. The percentage of growth reductions caused by fungicides in comparison with the fungicide free control were subjected to probit transformation. ED_{50} (effective dose inhibiting

50% of mycelial growth) values were then calculated using regression analysis of transformed growth reductions caused by fungicides and „ln" of fungicide concentrations. ED_{50} values thus obtained were compared with ANOVA and correlation analysis.

RESULTS

Different isolates of *Septoria tritici* highly significantly influenced ED_{50} levels. There were highly significant differences between mean levels of ED_{50} among the fungicides tested (Table 1).

ED_{50} values were significantly lower for metconazole than for flusilazole. LD 50 for Azoxystrobin was highest in *S. tritici* population; approximately 38% and 27% higher in comparison with metconazole and flusilazole respectively (Table 2). There were found the highest variability in the reaction of particular isolates to azoxystrobin characterized by s_x level.

Table 1. Analysis of variance for ED_{50} of different fungicides in *Septoria tritici* populations

Source of variation	d.f.	Mean square	Significance
ED 50 isolate	51	4.526	hs
ED 50 fungicide	2	5.573	hs
RESIDUAL	414	1.217	

Note: hs is significant at P = 0.01

Table 2. Multiple range analysis for ED_{50} of different fungicides

Fungicide	Mean ED_{50} (s_x) (n = 57)	Homogeneous Groups at 0,01
	Septoria tritici	
metconazole	0.61 (0.28)	A
flusilazole	0.72 (0.25)	B
azoxystrobin	0.98 (0.81)	C

Note: Differences significant at P 0.01

Table 3. The correlation coefficients between reaction of 52 isolates to DMI and strobilurine fungicides

Correlated traits	Correlation coefficient	Significance
$ED_{50\ flusilazole}$: $ED_{50\ metconazole}$	0.39	hs
$ED_{50\ flusilazole}$: $ED_{50\ azoxystrobin}$	-0.01	ns
$ED_{50\ metconazole}$: $ED_{50\ azoxystrobin}$	0.04	ns

Note: hs is significant at P 0.01 ns is non significant correlation

Table 4. Multiple range tests of *Septoria tritici* isolates which showed LD 50 to azoxystrobin higher than 0.98 (mean of experiment)

Isolate No.	Origin	Mean LD 50	Homogeneous Groups
708/02	Rychnov n. Kněžnou	1.0	a
574/02	Dobronín	1.0	a
562/02	Sovínky	1.0	a
19/02	Břečkov	1.0	a
530/02	Přepychy	1.14	b
443/02	Humburky	1.17	b
570/02	Brniště	1.20	b
525/02	Lukavice	1.21	b
166/02	Tršice	1.51	c
447/02	Libčany	1.56	c
468/02	Úsov	1.58	c
819/02	Žďár n. Metují	1.62	c
470/02	Úsov	2.19	d
165/02	Tršice	3.02	d
449/02	Libčany	3.06	d
371/02	Hrotovice	15.35	e

Note: Note: Differences significant at P 0.01

The results of correlation analysis are summarized in Table 3. Correlation coefficient was highly significant for ED 50 of both triazoles tested. Correlation coefficients were not signficant for LD 50 in comparison of triazoles and strobilurine fungicide suggesting independent reactions of particular isolates that were assessed.

16 isolates showed LD 50 for azoxystrobine higher than mean of the experiment. There were found two times higher LD 50 in isolate 470/02, three times higher in isolates 165/02 and 449/02 and fifteen times higher in isolate 371/02.

DISCUSSION

The risk of fungal resistance development to DMI has been ascertained in different pathogens. Some parameters pre-dispose pathogens to rapid change in susceptibility to DMI (Köller and Scheinpflug, 1987) including: single-site mode of actions, easily obtained resistant laboratory mutants, extensive use of as broad-spectrum fungicides. In recent years, considerable progress has been made in the development of resistance strategies (Urech, 1998). Such strategies aim to prolong the useful life of new and valuable fungicides and, thus, provide continued optimum disease control.

Strobilurin fungicides have become an integral part of disease-management programmes on a wide range of crops (Bartlett et al., 2002). The major reasons for the success of strobilurins have varied between individual active ingredients, but have consisted of one or more of the following: broad-spectrum activity and control of fungal isolates resistant to other fungicide modes of action.

A relatively important problem of the management of resistance to fungicides is the existence of the so-called „cross-resistance". Although DMI fungicides assessed in this experiment showed significant correlation in reaction type we can not speak about cross-resistance. Both active ingredients suppressed pathogen in growth of mycelium effectively with very low variability between particular isolates.

There were not significant correlation in reaction of isoloates to DMI fungicides (flusilazole and metconazole) and strobilurine fungicide azoxystrobin. It suggested an independent reaction of different isolates to fungicides of different chemical groups. This findings suggest that *Septoria tritici* population can be controled according with last guidelines of FRAC in the territory of the Czech Republic (anonymous, 2003). To protect strobilurins against the development of resistance in cereal pathogens they should be applied as tank mix or as a co-formulated mixture with an effective mixture partner, should be used in single or block applications in alternation with fungicides from a different cross-resistance group.

Although there is mentioned in the same source that in the Czech Republic has been assessed low or no resistance of *Septoria tritici* to strobilurins, we have found some isolates which showed many times higher LD 50 level (15 times at maximum) in comparison with the mean level of experiment. Interpretation of these findings is limited by the fact that the number of isolates was relatively low. On the other hand we found more resistant isolates in different growing regions of the Czech Republic and therefore could be expected that final frequency of resistance will be higher.

This type of test is sure to be limited by the fact that it is an *in vitro* test. Data from *in vivo* experiments from farm fields are necessary to fully interpret the results of the sensitivity test (Nuninger-Ney and Staub, 1991).

There seems to be general agreement that low numbers of fungicide-resistant strains exist before the introduction of a new fungicide. The frequency of resistant genotypes will increase under the selection pressure of the fungicides, and the entire population will shift to a new equilibrium (Köller and Scheinpflug, 1987).

Within integrated plant protection, leaf diseases of cereals are often suppressed with fungicides. Search for changes in reaction to the broad spectrum fungicides can help to detect the development of possible shift of resistance and to correct the advisory recommendations to farmers as early as possible.

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Streszczenie

Badania odporności grzyba *Septoria tritici* na kilka fungicydów na terenie Republiki Czech

Na podstawie redukcji średnicy kolonii grzyba *Septoria tritici* oceniano odporność populacji grzyba na dwa fungicydy z grupy IBE i jeden fungicyd strobilurynowy. 57 szczepów (izolatów) *Septoria tritici*, zebranych z różnych rejonów Republiki Czech, testowano „*in vitro*" na szalkach Petriego z agarem z dodatkiem fungicydów w różnych koncentracjach. Dla substancji aktywnych: flusilazol (Capitain), metconazol (Caramba) i azoxystrobina (Amistar) wyznaczono średnią wartość EC_{50}. Wartość EC_{50} dla metconazolu była niska. Obserwowano bardzo wysoką korelację reakcji szczepów patogena na metconazol i flusilazol. Kilka z badanych izolatów wykazywało wyraźnie wyższą odporność na metconazol. Poszukiwania odporności na fungicydy w populacjach *S. tritici* na terenie Czech będą kontynuowane.