Sensitivity Behaviour of *Microdochium nivale* Isolates to some DMI-Fungicides Commonly used in the Czech Republic*

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Abstract

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Reduction of radial mycelial growth by DMI-fungicides was evaluated in a *Microdochium nivale* population. In an *in vitro* assay 28 isolates of *M. nivale* were grown on agar plates containing different concentrations of fungicides and ED_{50} values were calculated. The fungicides were: metconazole, epoxiconazole, prochloraz and tebuconazole. ED_{50} values for prochloraz and epoxiconazole were very low. There were highly significant differences between mean levels of ED_{50} of the fungicides, but no significant differences between those of the isolates. The poor correlation between isolates and different fungicides suggests independent reactions of particular isolates to different DMI-fungicides. The data will be used in a continuing survey of resistance development in the *M. nivale* population of the Czech Republic. This research will be expanded for other azoles and fungicides with different modes of action.

Key word: *Microdochium nivale*; resistance to DMI-fungicides; ED_{50}; epoxiconazole; metconazole; tebuconazole; prochloraz

Microdochium nivale (Fr.) Samuels and I.C. Hallet (syn. *Fusarium nivale* Ces. ex. Berl. and Voglino) is a causal agent of pink snow mold of winter cereals. *Microdochium nivale* is distributed worldwide, especially in cold regions of the Northern Hemisphere, Australia and New Zealand (MCBEATH *et al.* 1993). It has been identified as one of the most common pathogens of wheat in Western Europe (PARRY 1990), and is one of the most important causal agents of foot rot disease of cereals together with *Pseudocercosporella herpotrichoides, Gaeumannomyces graminis,* and *Rhizoctonia cerealis.* Leaf blotch and ear blight can occur in adult plants when conditions are cool and humid in spring and early summer of the vegetation period (COOK 1981).

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

The risk of development of fungal resistance to DMI has been ascertained in different pathogens. Some parameters pre-dispose pathogens to rapid change in susceptibility to DMI including: single-site mode of actions, extensive use of DMI as broad-spectrum fungicides, high rate of mutability as demonstrated by easily obtained resistant mutants in the laboratory (KÖLLER & SCHEINPFLUG 1987). 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 to thus provide continued optimum disease control.

The initial work on methods to determine the sensitivity of *M. nivale* (*in vitro*, *in vivo*) has been completed (SCHEINPFLUG 1998). The resistance of *M. nivale* to dicarboximide and benzimidazole fungicides was studied (RESSELER & BUCHENAUER 1988). Some DMI-fungicides, carboxylic acid anilide derivatives, iprodione, maneb, guezatine were equally effective against carbendazim-resistant and sensitive strains (HARTKE & BUCHENAUER 1984). In tests of 16 fungicides in potato dextrose agar the myce-

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lial growth of *M. nivale* was inhibited by fenpropimorph and prochloraz, but sensitivity to some other fungicides varied among the isolates (MEUNIER *et al.* 1983). However, there is a lack of information concerning the resistance of the *M. nivale* population in the Czech Republic. Therefore, the sensitivity behaviour of the wild-type pathogen population to fungicides has been recognized as the first step of this type of regular survey. The "baseline data" of the reaction to four DMI-fungicides (prochloraz and tebuconazole which have been used for a long time, and metconazole and epoxiconazole that were registered during the last 3 years) were compared and expressed as ED_{50} values.

MATERIALS AND METHODS

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

Sample Collection: Wheat plants with typical pink snow mold symptoms were collected during early spring in 1997 from 28 locations in North and Central Moravia. Leaf sheaths with *M. nivale* symptoms were cut into pieces (2–3 mm) and surface sterilized in a 1% sodium hypochlorite solution for 3 min. The segments of plant tissue were then rinsed in sterile water, dried between two sheets of sterile filter paper and placed on potato-dextrose agar (PDA) in Petri dishes.

Three-day incubation in the dark at 21°C was followed by 4-day exposure to continuous NUV light at 12°C. Three to four cultures were recovered from each location. After species identification, 28 single spore isolates from different locations were transferred to PDA, incubated for 7 days in the dark at 21°C and used for the following tests.

Sensitivity Assay: Petri dishes containing PDA with or without fungicides were prepared. The four DMI-fungicides used were metconazole, epoxiconazole, prochloraz, and tebuconazole at the following rates 0.0, 0.1, 0.25, 0.5, 1.0, 2.0, 5.0 and 10.0 µg/ml (ppm). The test included two replications of each concentration/isolate/fungicide combination as well as two replications of the fungicide – free controls.

The radial mycelial growth was measured after incubation for 7 days in the dark at 21°C. The percentage of growth reduction caused by a fungicide in comparison with the fungicide – free control was subjected to probit transformation. ED_{50} (effective dose inhibiting mycelial growth by 50%) values were then calculated using regression analysis of transformed growth reductions caused by fungicides and "ln" of fungicide concentrations. The ED_{50} values were compared with ANOVA and correlation analysis.

RESULTS

The data obtained by calculating ED_{50} levels are summarized in Fig. 1. Very low ED_{50} levels were assessed for prochloraz and epoxiconazole.

There were highly significant differences between mean levels of ED_{50} of the DMI-fungicides tested. The isolates of *M. nivale* did not significantly differ in their ED_{50} levels of each individual fungicide.

Table 1. Analysis of variance for ED_{50} of different fungicides in *Microdochium nivale* population

	d.f.	Mean square	Significance
ED_{50} isolate	27	0.371	ns
ED_{50} fungicide	3	23.476	**
Residual	81	0.333	

** significant at 0.01; ns – non-significant

ED_{50} values were significantly lower for prochloraz and epoxiconazole than for metconazole and tebuconazole (Table 2). The highest mean ED_{50} was found for tebuconazole; approximately 50% and 90% higher in comparison with metconazole and prochloraz, respectively.

Table 2. Multiple range analysis for ED_{50} of different fungicide in *Microdochium nivale* population

Fungicide	Mean ED_{50} (n = 28)	Homogeneous groups at 0.01
Prochloraz	0.177	A
Epoxiconazole	0.257	A
Metconazole	1.084	B
Tebuconazole	1.930	C

The results of correlation analysis are summarized in Table 3. Correlation coefficients were not significant, suggesting independent reactions of particular isolates to the four DMI-fungicides.

Table 3. Correlation coefficients between reaction of *Microdochium nivale* isolates to DMI-fungicides

Correlated traits	Correlation coefficient
$ED_{50\ tebuconazole}$: $ED_{50\ metconazole}$	0.12
$ED_{50\ tebuconazole}$: $ED_{50\ epoxiconazole}$	–0.27
$ED_{50\ tebuconazole}$: $ED_{50\ prochloraz}$	0.03
$ED_{50\ prochloraz}$: $ED_{50\ metconazole}$	–0.35
$ED_{50\ prochloraz}$: $ED_{50\ epoxiconazole}$	–0.20
$ED_{50\ metconazole}$: $ED_{50\ epoxiconazole}$	0.03

DISCUSSION

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 by the fungicides, and the entire population will shift to a new equilibrium (KÖLLER & SCHEINPFLUG 1987).

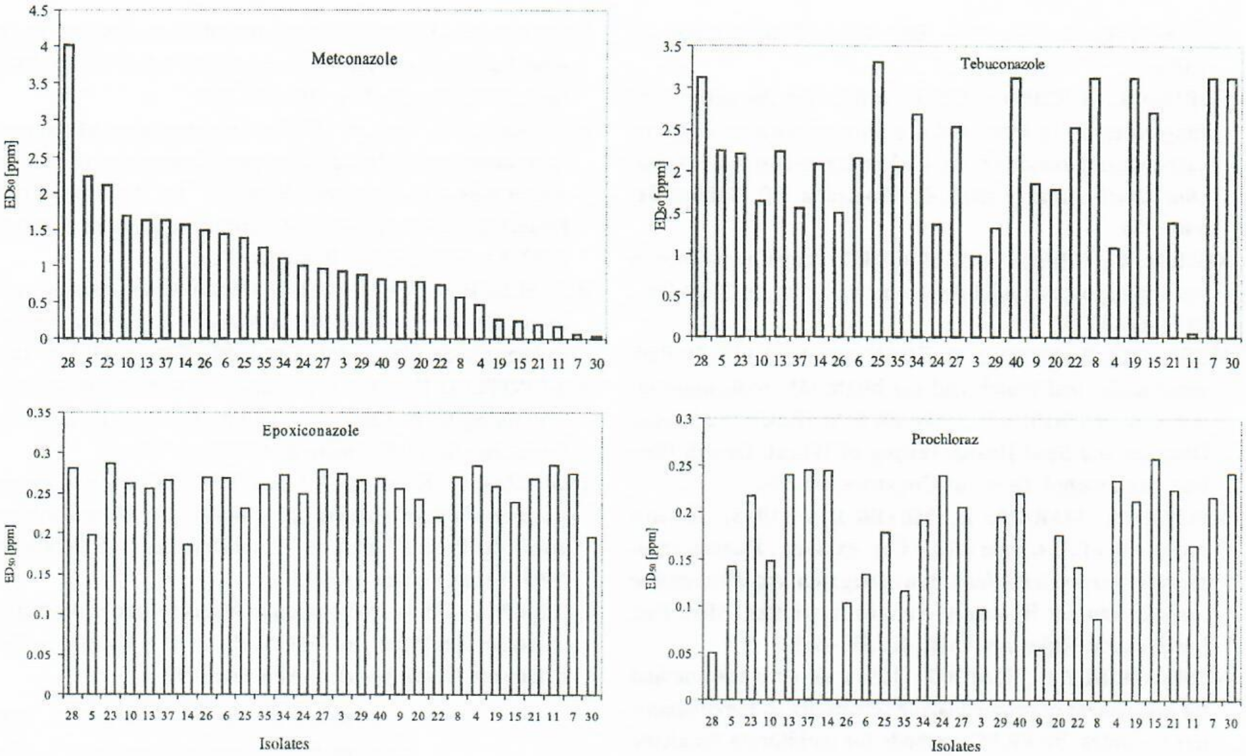

Fig. 1. ED_{50} of tested fungicides against isolates of *Microdochium nivale*

Different pathogens have been screened for resistance to fungicides on grown cereals. Regular surveys of resistance are common practice e.g., in powdery mildew (*Blumeria graminis*) and eyespot (*Pseudocercosporella herpotrichoides*) (FLETCHER & WOLFE 1981; CAVELIER & LARDIER 1997; POLIŠENSKÁ & VEJL 1997).

Microdochium nivale has become one of the most serious causal agents of foot rot disease of cereals during recent years. This may be due to the susceptibility of current cultivars (TVARŮŽEK & HRABALOVÁ 1997) as well as weather conditions favourable for pathogen development. Within integrated plant protection, foot rot diseases are often suppressed with fungicides. The search for changes in reaction to the broad spectrum fungicides can help to detect possible changes of resistance and to correct the recommendations to farmers as early as possible.

A relatively important problem of the management of resistance to fungicides is the existence of so-called "cross-resistance". None of the fungicides assessed here showed this reaction. This suggested an independent reaction of different populations to fungicides of the same chemical group. An interpretation of these findings is limited by the fact that the fungicides used in this experiment have not been registered for the same length of time in the Czech Republic and, consequently, their selection pressure was not comparable.

The data obtained will be used as the starting point of regular surveys. In the case of metconazole and tebuconazole we found strains that can be used as resistant standards, others as susceptible ones. On the other hand, the variation in isolate reaction to prochloraz and epoxiconazole was very low; these two compounds also had high toxicity for particular strains of the pathogen.

The usefulness of the type of test used here is certainly limited by the fact that it is an *in vitro* test. Data from *in vivo* experiments on farm fields are necessary to fully interpret the results of such sensitivity tests (NUNINGER-NEY & STAUB 1991).

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Souhrn

TVARŮŽEK L., KRAUS P., HRABALOVÁ H. (2000): **Rezistence izolátů *Microdochium nivale* k DMI-fungicidům používaným v České republice.** Plant Protect. Sci., **36**: 7–10.

Byla hodnocena redukce radiálního růstu mycélia v populaci *Microdochium nivale* způsobená DMI-fungicidy. V podmínkách *in vitro* bylo pěstováno 28 izolátů *M. nivale* na agarech obsahujících různé koncentrace fungicidů. Byla zjišťována hodnota ED_{50} jednotlivých izolátů v reakci na čtyři fungicidy ze skupiny DMI: metconazole, epoxiconazole, prochloraz a tebuconazole. Hodnoty ED_{50} pro prochloraz a epoxiconazole byly velmi nízké. Byly zjištěny vysoce průkazné rozdíly mezi průměrnými hodnotami ED_{50} pro jednotlivé fungicidy. Rozdíly mezi izoláty nebyly průkazné. Nízká a neprůkazná korelace ED_{50} izolátů k různým fungicidům potvrzuje nezávislou reakci. Nebyla prokázána cross rezistence. Získaná data budou využita při pravidelném vyhodnocování stavu rezistence k DMI-fungicidům v populaci *M. nivale* na území České republiky. Tento program bude rovněž rozšířen o další azoly a fungicidy s rozdílným směrem účinku na patogena.

Klíčová slova: *Microdochium nivale*; rezistence k DM-fungicidům; ED_{50}; epoxiconazole; metconazole; tebuconazole; prochloraz

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