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THE CHEMICAL CONTROL OF *ASCOCHYTA* LIB., A FUNGAL PATHOGEN OF MONOCOTYLEDONOUS PLANTS*

Tomasz Kosiada, Lidia Irzykowska

Department of Phytopathology, Poznan University of Life Sciences

kosiada@up.poznan.pl

Abstract. Fungi from the genus *Ascochyta* are usually facultative saprotrophs, but they may also cause diseases in mono- and dicotyledonous plants. In this study the effect of fungicides on mycelium growth in fungi infecting monocotyledonous plants (*A. agrostis, A. avenae, A. brachypodii, A. desmazieri, A. digraphidis, A. ducis-aprutii, A. festucae, A. graminea, A. hordei, A. hordei* var. *americana, A. hordei* var. *europea, A. hordei* var. *hordei, A. melicae, A. skagwayensis, A. sorghi, A. stipae, A. zeicola*) was assessed. Several fungicides were tested, which have been recommended in cereal protection programmes. Fungicides tested included azoxystrobin, benomyl, chlorothalonil, mancozeb, thiophanate methyl, prochloraz: tebuconazol: proquinazid (8:4:1), propiconazole: cyproconazole (3.13:1), spiroxamine: tebuconazole: triadimenol (5.81:3.88:1), thiophanate methyl: tetraconazole (3.33:1). The inhibition of mycelial growth was dependent on a kind and preparation dose. Fungicides: prochloraz: tebuconazol: proquinazid (8:4:1) – Wirtuoz 520 EC, propiconazole: cyproconazole (3.13:1) – Artea 330 EC, spiroxamine: tebuconazole: triadimenol (5.81:3.88:1) – Falcon 460 EC exhibited the most efficacious action, for all examined fungi EC_{50} was <1 mg·dm^{-3}.

Key words: *Ascochyta*, cereal diseases, fungicide

INTRODUCTION

Among fungal pathogens infecting cereals, species belonging to *Ascochyta* genus are poorly known. *Ascochyta* species occur on a wide range of hosts, including numerous cultivated plants and are usually facultative saprotrophs. Some species cause dicotyledonous plant diseases of economic relevance worldwide [Melnik et al. 2000]. *Ascochyta* species infecting legumes are host-specific [Hernandez-Bello et al. 2006] and attack all above ground parts of the plant. Approximately 80 species are known that can cause Ascochyta blight [Galloway and MacLeod 2003, Peever et al. 2007, Rajakumar et al. 2005].

In case of monocotyledonous plants *Ascochyta* spp. are considered to be rather weak pathogens so far. There is hardly any information concerning their harmfulness for cereals. The occurrence of 18 fungal species from the genus has been reported on cereals and grasses [Melnik 2000, Punithalingam 1979]. The importance of fungi from the genus *Ascochyta* may be considerable due to the potential for cereal infestation, particularly wheat and barley [Braithwaite et al. 1998, Bockus et al. 2010, Mathre 1997, Perelló and Moreno 2003]. Successful invasion by pathogens depends as much on their ability to utilize the available nutrient sources as on their ability to penetrate plant tissues and evade defensive mechanisms. Development of leaf spotting, yellowing and necrosis of lower leaves is favored by high humidity, and leaves in contact with

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soil. Lesions are round, oval or irregular, 3–11 mm in diameter, sometimes with rings. There are rarely more than a few lesions on an individual plant. Fungi are harbored on plant debris.

Ascochyta leaf spot is often overlooked in association with other leaf spot diseases. *A. avenae* was detected in Mid Canterbury. Its effects are similar to a number of other *Ascochyta* species which cause late season leaf spotting on wheat in New Zealand [Braithwaite et al. 1998]. Moreover, the disease has been found on barley in Japan, Korea, Europe, Argentina and the United States as a pathogen of minor economic importance [Scharen and Krupinsky 1971, Perelló and Moreno 2003]. However, pathogens evolve under pressure of environmental agents. In agricultural ecosystems, environmental changes may include resistant cultivars, applications of fungicides, irrigation, crop rotation and others [McDonald 1997]. It is well known that applications of fungicides impose strong directional selection on pathogen populations and weak pathogen can become a serious problem.

Undoubtedly, *Ascochyta* species infecting cereals have not been sufficiently studied, above all with regard to their sensitivity to fungicides. One of the parameters defining the practical applicability of fungicides is the capacity to limit mycelium growth, expressed as a concentration causing a 50% growth inhibition (EC_{50}) in relation to the control. This parameter was used to determine the effectiveness of fungicide against many pathogens [Obanor et al. 2005, Wise et al. 2008, Weber and Hahn 2011].

The present study was undertaken to evaluate the sensitivity of *Ascochta* species infecting monocotyledonous plant to selected fungicides widely used in cereal protection programs.

MATERIAL AND METHODS

A set of 14 species from the *Ascochyta* genus were studied. Fungal isolates came from the plant pathogen collection of the Department of Phytopathology, Poznan University of Life Sciences in Poland (collected in 2008–2010) and Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre (denoted by the letter C).

Analyses were carried out using different fungicides and their mixtures in commercially available formulations (Tab. 1). All the preparations were used at concentrations of 10000, 1000, 100, 10 and 1 $mg \cdot dm^{-3}$ one active substance or a total of active substances included in the preparation, by adding an appropriate amount of preparation to potato dextrose agar medium (PDA; Merck KGaA, Darmstadt, Germany). 5 mm discs of PDA overgrown by 14-day-old mycelium of the particular isolate were placed on such prepared Petri dishes. Mycelium growth of isolates was measured after two and seven days of culture on PDA medium at temperatures of 20°C and, in four replicates of each. The radial mycelial growth per day was then calculated. The percentage of mycelium growth inhibition as a result of fungicide activity was calculated, where 0% denotes a lack of growth inhibition (the control), while 100% denotes complete growth inhibition. The value of EC_{50} was calculated using a comparison of growth inhibition closest to 50% for a specific fungicide concentration. If all the applied fungicide concentrations caused a 100% mycelium growth inhibition, EC_{50} was given as <1. Laboratory tests were performed in 2011.

A two-way analysis of variance (ANOVA) was conducted, with fungicides used and fungal isolates as factors. Collected data were subjected to a multivariate statistical analysis – Principal Component Analysis. Statistical calculations were performed using Statistica v.8 software.

Table 1. Fungicides evaluated in the experiments

Active compound	Concentration	Trade name
azoxystrobin	250 g·dm^{-3}	Amistar 250 SC
benomyl	500 g·kg^{-1}	Benlate 50 WP
chlorothalonil	500 g·dm^{-3}	Gwarant 500 SC
mancozeb	75%	Dithane NeoTec 75 WG
prochloraz tebuconazole proquinazid	320 g·dm^{-3} 160 g·dm^{-3} 40 g·dm^{-3}	Wirtuoz 520 EC
propiconazole cyproconazole	250 g·dm^{-3} 80 g·dm^{-3}	Artea 330 EC
spiroxamine tebuconazole triadimenol	250 g·dm^{-3} 167 g·dm^{-3} 43 g·dm^{-3}	Falcon 460 EC
thiophanate methyl	500 g·dm^{-3}	Topsin M 500 SC
thiophanate methyl tetraconazole	233 g·dm^{-3} 70 g·dm^{-3}	Yamato 303 SE

RESULTS

Results of the biotest indicated significant differences between *Ascochyta* species in respect to their growth in response to selected fungicides. Calculated EC_{50} values highly varied and depended on the fungal species and fungicide (Tab. 2). In case of propiconazole: cyproconazole (3.13:1) and spiroxamine: tebuconazole: triadimenol (5.81:3.88:1) a very strong inhibition of mycelium growth was observed, as at the lowest analysed fungicide concentration (1 mg·dm^{-3}) it was close to 100%. Chlorotalonil, benomyl, thiophanate methyl and azoxystrobin reduced fungal growth the least effectively, as for them mean value of EC_{50} was 141, 131, 92 and 73 mg·dm^{-3}, respectively.

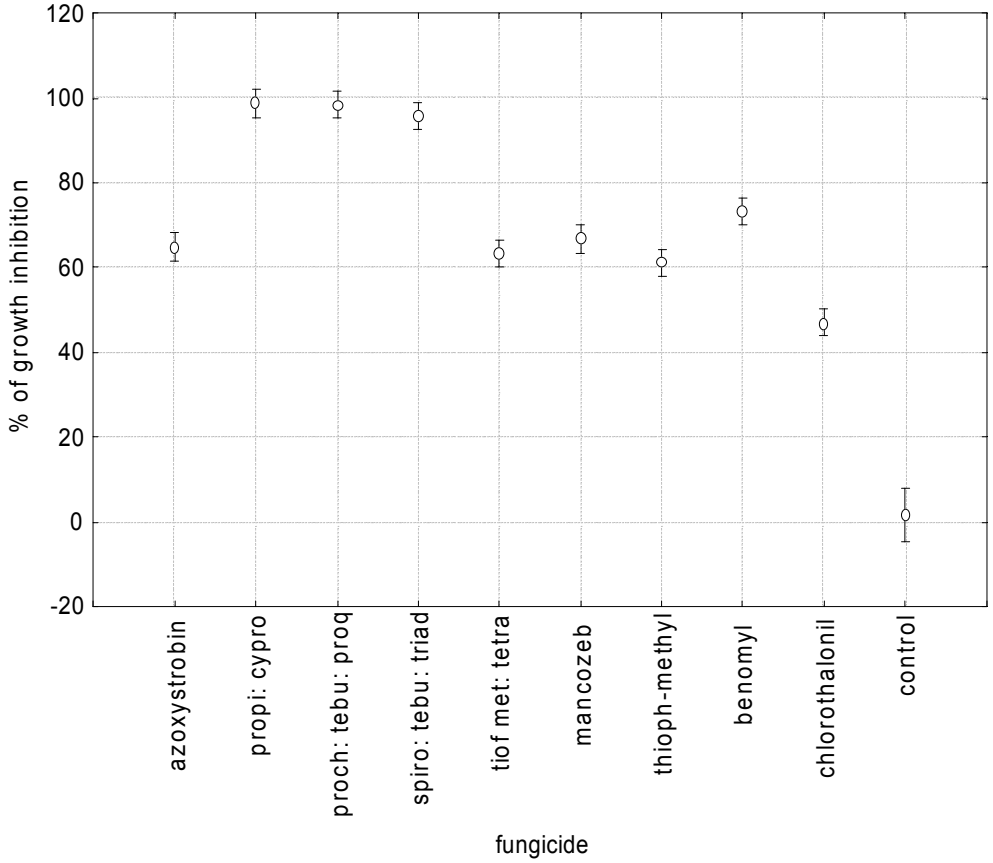
Some isolates were characterized by a considerable resistance to most applied fungicides, for *A. digraphidis* (1968b) mean EC_{50} was 375 mg·dm^{-3}, while for *A. stipa*e (1811) mean EC_{50} was 313 mg·dm^{-3}. Some fungi exhibited high sensitivity to used fungicides, for *A. ducis-aprutii* (1915), *A. hordei* (1684), *A. hordei* var. *europea* (C817.84), *A. melicae* (793c) and *A. skagwayensis* (C110124) EC_{50} was below 5 mg·dm^{-3}. A similar fungal growth inhibition close to 100% was found for spiroxamine: tebuconazole: triadimenol (5.81:3.88:1), prochloraz: tebuconazole: proquinazid (8:4:1) and propiconazole: cyproconazole (3.13:1). Slightly lower mycelium

Table 2. EC_{50} (mg·dm^{-3}) values for in vitro growth of fungi *Ascochyta* sp.

Species	Isolate	Fungicide EC_{50} (mg·dm^{-3})								
		azoxystrobin	benomyl	chlorothalonil	mancozeb	prochloraz: tebuconazol: proquinazid (8:4:1)	propiconazole: cyproconazole (3.13:1)	spiroxamine: tebuconazol: triadimenol (5.81:3.88:1)	thiophanate methyl	thiophanate methyl: tetraconazole (3.33:1)
A. agrostis	C758.97	1.1	9.1	166.1	10.9	<1	<1	<1	61.6	9.8
A. avenae	1982	94.9	1.2	1.5	10.9	<1	<1	<1	7.5	16.5
A. avenae	C811.84a	10.6	<1	82.9	12.0	<1	<1	<1	12.1	13.1
A. avenae	C811.84b	86.9	1.5	1.1	12.5	<1	<1	<1	6.8	13.3
A. brachypodii	1523a/2	152.8	1.1	11.5	9.8	<1	<1	<1	<1	<1
A. desmazieri	C247.79	<1	3.4	190.0	13.6	<1	<1	<1	13.7	8.6
A. digraphidis	1968b	1005.1	1401.4	184.3	<1	<1	<1	<1	235.8	171.0
A. ducis-aprutii	1915	<1	<1	10.0	10.2	<1	<1	<1	<1	7.9
A. festucae	C689.97	10.1	1.6	109.8	8.4	<1	<1	<1	24.1	<1
A. graminea	C586.79	<1	<1	140.7	17.9	<1	<1	<1	5.7	11.4
A. hordei	1684	<1	<1	12.3	9.3	<1	<1	<1	<1	9.0
A. hordei var. *americana*	C814.84	<1	<1	88.7	15.5	<1	<1	<1	<1	11.4
A. hordei var. *europea*	C817.84	<1	2.0	10.9	0.9	<1	<1	<1	6.1	8.2
A. hordei var. *hordei*	C878.72a	<1	2.1	94.7	28.6	<1	<1	<1	6.4	18.2
A. melicae	793c	<1	0.9	11.5	<1	<1	<1	<1	7.9	15.7
A. skagwayensis	C110124	<1	1.7	<1	<1	<1	<1	<1	8.2	15.3
A. sorghi	C622.68	<1	4.7	117.5	42.2	<1	<1	<1	1.7	11.0
A. stipae	1811	9.5	1053.2	113.8	1.0	<1	<1	<1	1323.5	<1
A. zeicola	1937	10.1	<1	1328.9	19.1	<1	<1	<1	21.6	14.3

growth inhibition of little less than 73% was caused by benomyl. Growth inhibition from 61 to 66% was caused by the application of thiophanate methyl, thiophanate methyl: tetraconazole (3.33:1), azoxystrobin and mancozeb. The weakest mycelium growth inhibition was caused by chlorothalonil (Fig. 1). Among analyzed isolates no considerable differences were observed in terms of growth inhibition in relation to the control, except for *A. digraphidis* (1968b), which percentage of growth inhibition was lowest (Fig. 2).

The application of Principal Component Analysis made it possible to place in a 2D space, explaining 94% variation, points representing used fungicides. Three groups of points can be observed. The first one located very close to one another represented propiconazole: cyproconazole (3.13:1); prochloraz: tebuconazole: proquinazid (8:4:1); spiroxamine: tebuconazole:



Applied name abbreviations:
propi: cypro- propiconazole: cyproconazole (3.13:1); proch: tebu: proq- prochloraz: tebuconazole: proquinazid (8:4:1); spiro: tebu: triad- spiroxamine: tebuconazole: triadimenol (5.81:3.88:1); tioph met: tetra- thiophanate-methyl: tetraconazole (3.33:1)

Fig. 1. Percentages of mycelium growth inhibition depending on the applied fungicides

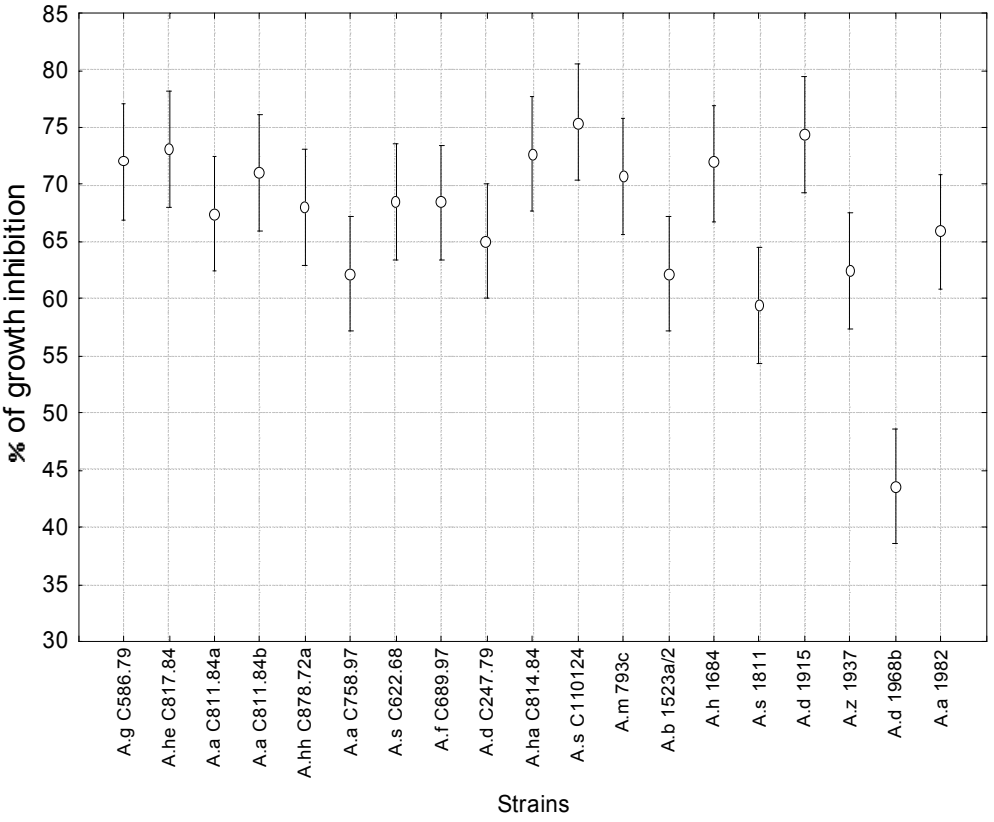


Fig. 2. Percentages of mycelium growth inhibition in isolates of Ascochyta spp.

triadimenol (5.81:3.88:1). The second group of points located close to one another represented azoxystrobin, thiophanate methyl and benomyl, while the third group represented mancozeb, chlorothalonil, thiophanate methyl: tetraconazole (3.33:1), which was located closest to the control (Fig. 3). Fungicides, which on the graph in the form of points are located close to one another, are characterized by a similar action. The close location of the first group of points in relation to the control indicates weak action of these fungicides.

DISCUSSION

There is hardly any published data on fungicide effectiveness against *Ascochyta* species examined in this study. However, it needs to be assumed that they exhibit a similar sensitivity to fungicides as other species from the genus *Ascochyta*, or other closely related species. Similarly as for most isolates investigated in this study, when sensitivity of *A. rabiei* was assessed, EC_{50} for azoxystrobin and benomyl were equal 0.59 $\mu g \cdot mL^{-1}$ and 2.3 $\mu g \cdot mL^{-1}$ (1 $\mu g \cdot mL^{-1} = 1\ mg \cdot dm^{-3}$),

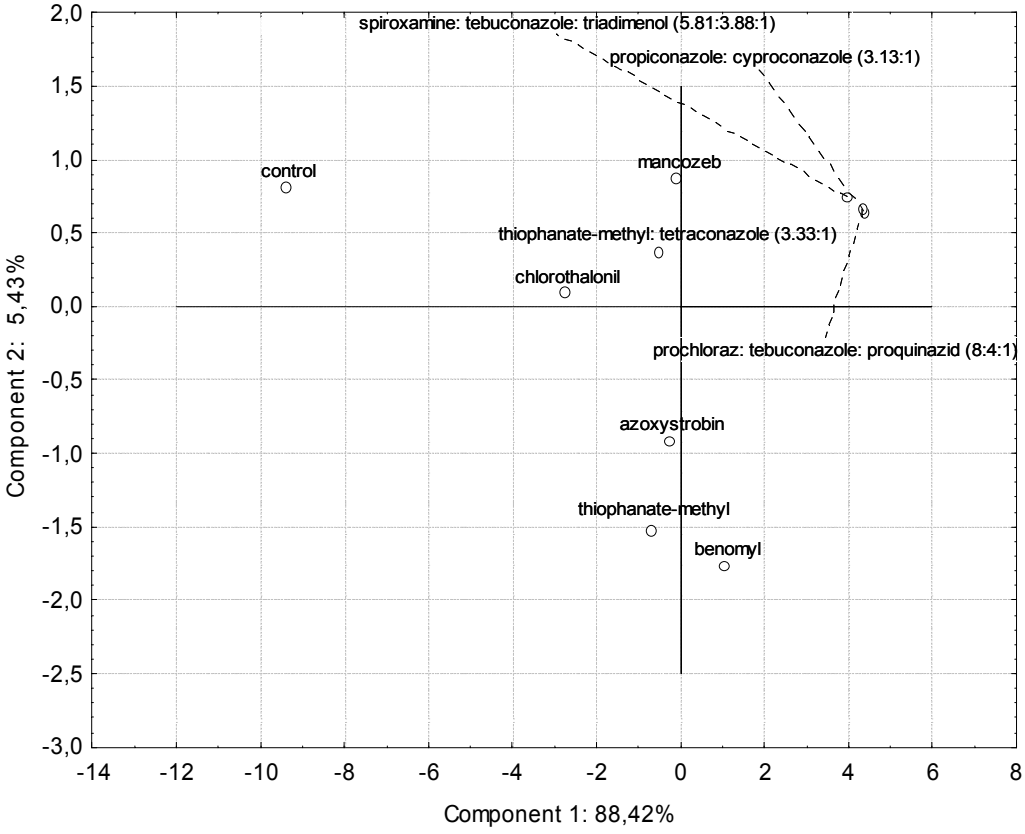


Fig. 3. Configuration of points representing fungal growth inhibition as a result of action of fungicides in the system of two Principal Components

respectively [Demirci et al. 2003). Chlorotalonil and mancozeb displayed even higher EC_{50} value (37.12 µg·dm^{-3} and 51.18 µg·dm^{-3}) for all analyzed isolates of *Ascochyta* spp. than for *A. rabiei*. Similar EC_{50} values were recorded for *A. pinodes*, for chlorotalonil amounting to 75 mg·dm^{-3} [Gorfu and Sangchote 2003]. In case of *Ascochyta chrysanthemi*, *A. lycopersici* and *A. pisi* EC_{50} for benomyl ranged from 1 to 5 mg·dm^{-3}, for *A. pinodes* it was 5–10 mg·dm^{-3} [Bollen and Fuchs 1970]. For most analyzed fungi of *Ascochyta* sp. EC_{50} for benomyl ranged from <1 to 9 mg·dm^{-3}, except for *A. digraphidis* (1968b) and *A. stipae* (1811), which are resistant to benomyl. Bollen and Fuchs [1970] classified fungi based on EC_{50} value: at EC_{50} >100 mg·dm^{-3} – resistant fungi, EC_{50} 10–100 mg·dm^{-3} – tolerant, EC_{50} 1–10 mg·dm^{-3} – sensitive and EC_{50} 0.1–1 mg·dm^{-3} – very sensitive fungi.

A high variation in sensitivity between isolates and species may indicate the occurrence of resistance of fungi to used fungicides. In studies on *A. rabiei* it was found that 35% of the isolates were insensitive to chlorothalonil, while 53% were insensitive to mancozeb. In the populations studied some isolates were insensitive to two or even three fungicides [Chang et al. 2007]. The resistance of several *Ascochyta* species to fungicides used in cereal growing may

explain more frequent isolation of these fungi from wheat and barley plants. In fungal population derived from cropped fields where fungicides were frequently applied, isolates insensitive to fungicides appeared [Chang et al. 2007, Sartori and Maringoni 2008, Weber et al. 2005]. This may indicate an occurrence of fungal populations resistant to some active compounds of fungicides in agricultural ecosystems. Consistently, Wise et al. [2011] observed an increase of EC_{50} values for *A. rabiei* in successive years of analyses, what can be explained by application of fungicides in successive years of cultivation. Moreover, significant variation was observed in the sensitivity of isolates according to the region of provenance [Chang et al. 2007, Sartori and Maringoni 2008, Wise et al. 2009].

To conclude, obtained results revealed that the inhibition of *Ascochyta* mycelium growth was directly proportional to increase of fungicides concentration. The only exception was azoxystrobin for which increase of concentration exert quite weak influence on mycelium growth inhibition. A similar dependence for this fungicide was recorded earlier [Kosiada 2011, Sikora and Banachowska 2006] also for another strobilurin [Schmitz et al. 2006]. For *Phoma exigua* (a fungus closely related with the *Ascochyta* genus) EC_{50} recorded for trifloxystrobin (a fungicide from the strobilurin group) ranged from 500 to 5000 $mg \cdot dm^{-3}$ [Schmitz et al. 2006]. Although azoxystrobin inhibits mycelium growth of *Ascochyta* spp. weakly, remains a fungicide active at very low doses against a wide range of fungal pathogens. The mode of strobilurin action is based on inhibition of spore germination and mitochondrial respiration in fungi [Wong and Wilcox 2001].

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T. Kosiada, L. Irzykowska

WRAŻLIWOŚĆ GRZYBÓW RODZAJU *ASCOCHYTA* LIB. PORAŻAJĄCYCH ROŚLINY JEDNOLIŚCIENNE NA FUNGICYDY

Synopsis. Grzyby rodzaju *Ascochyta* są zazwyczaj fakultatywnymi saprotrofami, ale mogą również powodować choroby roślin jednoliściennych i dwuliściennych. W pracy badano wpływ fungicydów na wzrost grzybni grzybów porażających rośliny jednoliścienne (*A. agrostis, A. avenae, A. brachypodii, A. desmazieri, A. digraphidis, A. ducis-aprutii, A. festucae, A. graminea, A. hordei, A. hordei* var. *americana, A. hordei* var. *europea, A. hordei* var. *hordei, A. melicae, A. skagwayensis, A. sorghi, A. stipae, A. zeicola*). Przebadano kilka fungicydów, które były lub są zalecane w programach ochrony zbóż. Fungicydy: prochloraz: tebuconazol: proquinazid (8:4:1) – Wirtuoz 520 EC, propiconazole: cyproconazole (3.13:1) – Artea 330 EC, spiroxamine: tebuconazole: triadimenol (5.81:3.88:1) – Falcon 460 EC działały najlepiej, dla wszystkich badanych grzybów EC_{50} było <1 mg·dm^{-3}. W przypadku pozostałych fungicydów działanie było różne i zależało od rodzaju fungicydu i izolatu.

Słowa kluczowe: *Ascochyta*, fungicyd, wrażliwość