Dehydrogenases, urease and phosphatases activities of soil contaminated with fungicides

E. Jastrzębska, J. Kucharski

University of Warmia and Mazury in Olsztyn, Poland

ABSTRACT

A greenhouse experiment was performed to determine the effects of the fungicides containing cyprodinil as well as dimoxystrobin and epoxiconazole on the activity of soil enzymes: dehydrogenases, urease, acid phosphatase and alkaline phosphatase, depending on fungicide dose (0 – control, 1 – recommended dose, 10-fold dose and 100-fold dose), the method of soil management (soil cropped with spring barley cv. Start, and uncropped soil) and the time of fungicide action (14, 28, 42 and 56 days). The experiment was established on Eutric Cambisols with pH_{KCl} = 6.7. It was found that the enzymatic activity of the soil was affected primarily by such factors as fungicide dose, method of soil management and time of fungicide action, and to a much lesser degree by the type of fungicide. Fungicide contamination of the soil significantly inhibited the activity of dehydrogenases and urease, and produced a significant negative effect (100-fold dose) on spring barley yield. A higher activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase was recorded in the soil cropped with spring barley cv. Start.

Keywords: fungicides; cyprodinil; dimoxystrobin; epoxiconazole; soil contamination; dehydrogenases; urease; phosphatases

The transformation of natural ecosystems into agricultural ecosystems characterized by a low biodiversity, as well as the intensive development of farming systems, resulted in a large-scale application of crop protection chemicals. Fungicides are one of pesticide groups used for crop protection against pathogenic fungi. Fungicides contain one or several active substances, including, among others, benzimidazoles – mitosis inhibitors, azimino compounds and imidazoles inhibiting ergosterol biosynthesis, morpholines – inhibitors of biosynthesis of nucleic acids and ergosterol, and strobilurins – fungal respiration inhibitors (Jańczak et al. 2004).

Unix 75 WG and Swing Top 183 SC are recommended for crop protection against fungal pathogens belonging to *Ascomycetes*, *Basidiomycetes* and *Deuteromycetes*. Cyprodinil in the fungicide Unix 75 WG, and dimoxystrobin and epoxiconazole in the fungicide Swing Top 183 SC are biologically active substances in these preparations. They have a broad spectrum of activities, but differ in the mechanism of their effect on fungi. Cyprodinil is anilinopyrimidine that inhibits methionine biosynthesis through blocking cystathionine-β-lyase and secretion of hydrolytic enzymes by pathogenic fungi (Rosslenbroich and Stuebler 2000). Dimoxystrobin belongs to strobilurins – metabolites produced by fungi and myxobacteria: *Strobilurus tenacellus*, *Oudemansiella mucida*, *Myxococcus fulvus*, acting against fungi. Synthetic strobilurin, known as azoxystrobin, was produced following the isolation of a substance antagonistic against other fungi from the fungus *Strobilurus tenacellus*. Strobilurins block electron transport in the mitochondrial respiratory chain (Karadimos et al. 2005). Epoxiconazole is a derivative of azimino compounds, disturbing sterol biosynthesis. It is a very common component of modern plant protection chemicals, since it is effective even when applied at a low dose (Elmhalt 1992).

Pesticides enable to achieve higher crop yields (Ray et al. 2004, Pasquer et al. 2005, Valenciano et al. 2006), but at the same time negatively affect natural environment, including the soil environment, disturbing its homeostasis.

An analysis of soil enzymatic activity is one of microbiological indicators of soil quality (Winding et al. 2005). Enzymes participate in numerous biochemical processes occurring in the soil, and

– as shown by the results of studies – they are sensitive to all environmental changes caused by natural and anthropogenic factors (Trasar-Capeda et al. 2000). Enzymes are secreted by floral and faunal organisms, but most often they are produced by microorganisms. Soil analysis includes the determination of the activity of intracellular enzymes, enzymes found on the cell surface and free enzymes. Their activity is related to the physical properties of the soil, organic matter content and the mechanism of action (Winding et al. 2005).

Crop protection preparations, especially when applied in excess of recommended amounts, may cause a variety of negative environmental changes, reflected by yield decrease and inhibition of soil biological activity (Wyszkowska and Kucharski 2004). The aim of the study was to determine the effects of the fungicides Unix 75 WG and Swing Top 183 SC on the activity of soil enzymes and spring barley yield.

MATERIAL AND METHODS

A pot experiment was performed in four replications, in a greenhouse of the University of Warmia and Mazury in Olsztyn, on Eutric Cambisols developed from heavy loamy sand of A-horizon (1–15 cm). The soil had the following properties: pH_{KCl} = 6.7, hydrolytic acidity Hh – 9.0 mmol/kg of soil, organic carbon content C_{org} – 8.50 g/kg.

Prior to placing in polyethylene pots, soil samples weighing 3.2 kg were thoroughly mixed with macroelements, microelements and fungicides. The fertilization rate expressed as the weight of pure elements per unit area was as follows: macroelements (g/kg of soil): N – 0.12 [$CO(NH_2)_2$], P – 0.096 (KH_2PO_4), K – 0.12 (KH_2PO_4 + KCl), Mg – 0.02 ($MgSO_4.7\ H_2O$); microelements (mg/kg of soil): Zn – 5.0 ($ZnCl_2$), Cu – 5.0 ($CuSO_4.5\ H_2O$), Mn – 5.0 ($MnCl_2.5\ H_2O$), Mo – 5.0 ($Na_2MoO_4.2\ H_2O$), B – 0.33 (H_3BO_3). Two fungicides were applied: Unix 75 WG and Swing Top 183 SC (0 – control, 1 – dose recommended by the producer, 10-fold dose and 100-fold dose; 0.25 µl/kg soil dm of cyprodinil, 0.067 mg/kg soil dm of dimoxystrobin and 0.025 mg/kg soil dm of epoxiconazole, respectively). The soil in a half of the pots was cropped with spring barley cv. Start (15 plants per pot), and the soil in the other half of the pots remained uncropped. Over the entire experimental period (56 days) soil moisture content was 60% of the soil capillary water capacity.

Spring barley was harvested at the flowering stage. Soil samples were collected four times, at 14-day intervals (14, 28, 42 and 56 days) after seed sowing, to determine the activity of soil enzymes: dehydrogenases [Deh] as described by Öhlinger (1996), urease [Ure] as described by Alef and Nannipieri (1998), acid phosphatase [Pac] and alkaline phosphatase [Pal] as described by Alef et al. (1998).

The results were verified statistically by a four-factor analysis of variance, using Statistica software (StatSoft, Inc., 2003) and the Duncan's multiple range test. The coefficients of correlation between soil enzymatic activity, spring barley yield and variable experimental factors, were also calculated.

RESULTS AND DISCUSSION

In the present experiment soil enzymatic activity was modified by soil contamination by the fungicides Swing Top 183 SC and Unix 75 WG. Both fungicides had a comparable effect on enzymes despite the fact that they contained different active substances, characterized by varied mechanisms of action. The coefficients of correlation between the variable experimental factors, and the activity of particular enzymes and spring barley yield, suggest that the type of fungicide had a significant effect

Table 1. Correlation coefficients between variable factors in the experiment

Variable	Type of fungicide	Fungicide dose	Method of soil management	Time of fungicide action
Deh	0.03	–0.46**	–0.74**	0.70**
Ure	–0.09	–0.31**	–0.61**	0.79**
Pal	0.31*	–0.21	–0.62**	0.75**
Pac	0.12	–0.30*	–0.66**	0.73**
Yield	0.21	–0.80**	–	–

Deh = dehydrogenases, Ure = urease, Pal = alkaline phosphatase, Pac = acid phosphatase, Yield = spring barley yield; * and ** = statistically significant differences at $P < 0.05$ and $P < 0.01$, respectively

only in the case of alkaline phosphatase activity (Table 1). The other factors (fungicide dose, the method of soil management and the time of fungicide action) were significantly correlated with soil enzymatic activity.

Leaving aside the time of fungicide action, it may be concluded that increasing doses of both fungicides significantly inhibited the activity of dehydrogenases and urease, which was particularly noticeable in soil cropped with spring barley (Table 2). Acid phosphatase exhibited a similar response in soil contaminated by the fungicide Swing. Alkaline phosphatase activity was affected by the type of fungicide – it was significantly inhibited by Swing (especially when applied at high doses) and stimulated by Unix.

The very few papers dealing with the effects of fungicides on the enzymatic activity of soil concern preparations that contain other active substances than those analyzed in this study. Among a variety of soil enzymes tested by different authors, dehydrogenases, β-glucosidase (Monkiedje et al. 2002, Demenaou et al. 2004) and alkaline phosphatase (Monkiedje et al. 2002) were found to be the most sensitive to the fungicides penetrating into the soil. Chen et al. (2001) demonstrated that benomyl and captan inhibited the activity of dehydrogenases and acid phosphatase.

The activity of all tested enzymes was significantly affected by the method of soil management (Table 2). Growing spring barley had a particularly positive influence on dehydrogenases, whose activity was twofold higher in cropped soil, as compared with uncropped soil.

The time of fungicide action (Tables 3–6) was another factor that modified soil enzymatic activity. The activity of all enzymes was positively correlated with the time of fungicide action (Table 1), which means that it increased with time. As regards dehydrogenases (Table 3), a significant correlation between their activity and the time of fungicide action was recorded only in soil cropped with spring barley, which indicates a substantial effect of plant developmental stage on this enzyme. A similar

Table 2. Activity of enzymes in the soil cropped with spring barley (+ plant) and uncropped (– plant) contaminated with fungicides

Fungicide dose[k]	Deh		Ure		Pal		Pac	
	(cm^3 H_2/kg dm of soil/d)		(mg N-NH_4^+/kg dm of soil/h)		(mmol PNP/kg dm of soil/h)		(mmol PNP/kg dm of soil/h)	
	method of soil management							
	+ plant	– plant	+ plant	– plant	+ plant	– plant	+ plant	– plant
Swing Top 183 SC								
0	8.99	4.41	29.70	20.23	2.13	1.75	1.79	1.34
1	9.13	4.67	31.79	20.08	2.12	1.76	2.02	1.45
10	8.31	4.58	28.12	19.90	1.99	1.75	1.72	1.35
100	5.41	4.41	22.88	19.44	1.78	1.69	1.47	1.32
r	–0.61**	–0.06	–0.42*	–0.20	–0.58**	–0.26	–0.47**	–0.17
Unix 75 WG								
0	8.99	4.34	29.70	20.23	2.13	1.75	1.79	1.34
1	8.31	4.98	26.77	21.52	2.14	1.78	1.87	1.37
10	7.89	4.40	26.13	18.00	2.20	1.77	1.85	1.37
100	7.18	3.43	25.20	18.99	2.21	1.97	1.77	1.30
r	–0.31	–0.46*	–0.16	–0.26	0.10	0.57*	–0.05	–0.17
$LSD_{P<0.01}$	a = ns, b = 0.16, c = 0.11		a = 0.36, b = 0.51, c = 0.36		a = 0.02, b = 0.03, c = 0.02		a = 0.02, b = 0.03, c = 0.02	
	a × b = 0.22, a × c = 0.16,		a × b = 0.72, a × c = 0.51,		a × b = 0.04, a × c = 0.03,		a × b = 0.04, a × c = 0.03,	
	b × c = 0.22, a × b × c = 0.32		b × c = 0.72, a × b × c = 1.02		b × c = 0.04, a × b × c = 0.06		b × c = 0.04, a × b × c = 0.06	

[k]0 – control; 1 – recommended dose; 10-fold dose and 100-fold dose, Deh = dehydrogenases, Ure = urease, Pal = alkaline phosphatase, Pac = acid phosphatase, * and ** = statistically significant differences at $P < 0.05$ and $P < 0.01$, respectively; LSD = least significant differences, a = type of fungicide, b = fungicide dose, c = method of soil management, a × b, a × c, b × c, a × b × c = factor interaction, ns = non significant

Table 3. Activity of dehydrogenases ($cm^3 H_2$/kg dm/d) in the soil contaminated with fungicides

Fungicide dose[k]	Soil cropped with spring barley					Uncropped soil				
	time of fungicide action				*r*	time of fungicide action				*r*
	14	28	42	56		14	28	42	56	
Swing Top 183 SC										
0	5.34	9.17	11.32	10.14	0.80*	4.77	3.78	4.40	4.67	0.09
1	7.21	7.79	10.79	10.74	0.89**	4.58	4.42	4.53	5.15	0.57
10	5.94	7.60	9.87	9.83	0.46	4.76	4.35	4.17	5.04	0.19
100	4.70	4.15	6.23	6.54	0.76*	4.45	4.11	4.09	4.98	0.48
r	−0.68	−0.94**	−0.94**	−0.95**		−0.39	−0.06	−0.61	0.09	
Unix 75 WG										
0	5.34	9.17	11.32	10.14	0.80*	4.77	3.78	4.40	4.67	0.09
1	5.19	8.46	10.23	9.35	0.82**	5.31	4.18	4.66	5.75	0.29
10	5.25	7.75	9.93	8.64	0.80*	4.59	3.44	4.03	5.53	0.47
100	5.16	5.43	8.64	9.50	0.93**	3.80	2.66	2.69	4.55	0.30
r	−0.44	−0.91**	−0.73*	0.02		−0.76*	−0.77*	−0.89**	−0.58	
$LSD_{P<0.01}$	a = ns, b = 0.16, c = 0.11, d = 0.16, a × b =0.22, a × c = 0.16, a × d = 0.22 b × c = 0.22, b × d = 0.32, c × d = 0.32, a × b × c = 0.32, a × b × d = 0.45 a × c × d = 0.32, b × c × d = 0.45, a × b × c × d = 0.63									

d = time of fungicide action, for remaining explanation see Table 2

tendency, with some exceptions, was observed for urease activity (Table 4). The impact of time on alkaline phosphatase activity (Table 6) depends primarily upon soil management method. In soil cropped with spring barley the activity of enzymes increased with time, regardless of fungicide dose. The opposite (falling) tendency was observed in uncropped soil. The effects of fungicides on soil

Table 4. Activity of urease (mg $N\text{-}NH_4^+$/kg dm/h) in the soil contaminated with fungicides

Fungicide dose[k]	Soil cropped with spring barley					Uncropped soil				
	time of fungicide action				*r*	time of fungicide action				*r*
	14	28	42	56		14	28	42	56	
Swing Top 183 SC										
0	23.01	26.75	34.83	34.22	0.92**	18.31	22.04	20.11	20.47	0.38
1	20.01	26.36	40.86	39.91	0.93**	18.21	20.73	21.03	20.34	0.60
10	18.70	22.41	36.54	34.82	0.81**	18.00	20.29	20.50	20.82	0.75*
100	18.00	22.87	25.49	25.16	0.89*	16.53	20.13	20.07	21.03	0.83**
r	−0.68	−0.58	−0.91**	−0.91**		−0.78*	−0.55	−0.26	0.36	
Unix 75 WG										
0	23.01	26.75	34.83	34.22	0.92**	18.31	22.04	20.11	20.47	0.38
1	20.07	22.98	34.19	29.84	0.81**	18.21	20.00	24.58	23.29	0.87**
10	19.39	22.25	32.18	30.71	0.90**	18.27	18.35	22.33	13.06	−0.39
100	16.56	23.11	31.46	29.65	0.90**	18.64	19.13	21.75	16.45	−0.23
r	−0.83**	−0.27	−0.72*	−0.48		0.37	−0.39	−0.14	−0.36	
$LSD_{P<0.01}$	a = 0.36, b = 0.51, c = 0.36, d = 0.51, a × b = 0.72, a × c = 0.51, a × d = 0.72 b × c = 0.72, b × d = 1.02, c × d = 0.72, a × b × c = 1.02, a × b × d = 1.45 a × c × d = 1.02, b × c × d = 1.45, a × b × c × d = 2.05									

Explanation see Table 2

Table 5. Activity of acid phosphatase (mmol PNP/kg dm/h) in the soil contaminated with fungicides

Fungicide dose[k]	Soil cropped with spring barley					Uncropped soil				
	time of fungicide action				*r*	time of fungicide action				*r*
	14	28	42	56		14	28	42	56	
Swing Top 183 SC										
0	1.79	1.96	2.29	2.49	0.98**	1.78	1.74	1.70	1.76	−0.27
1	1.85	1.96	2.24	2.43	0.97**	1.92	1.74	1.67	1.69	−0.85**
10	1.88	1.94	2.10	2.05	0.37	1.86	1.73	1.73	1.68	−0.89**
100	1.74	1.75	1.80	1.83	0.71*	1.80	1.66	1.51	1.77	−0.24
r	−0.73*	−0.94**	−0.93**	−0.81**		−0.38	−0.96**	−0.93**	0.57	
Unix 75 WG										
0	1.79	1.96	2.29	2.49	0.98**	1.78	1.74	1.70	1.76	−0.28
1	1.77	2.00	2.21	2.58	0.98**	1.89	1.75	1.64	1.83	−0.37
10	1.92	2.00	2.21	2.65	0.94**	1.85	1.72	1.69	1.83	−0.16
100	2.08	2.03	2.16	2.56	0.83**	2.09	1.74	1.87	2.17	0.38
r	0.93**	0.41	−0.61	−0.05		0.82**	0.13	0.94**	0.98**	
$LSD_{P<0.01}$	a = 0.02, b = 0.03, c = 0.02, d = 0.03, a × b = 0.04, a × c = 0.03, a × d = 0.04 b × c = 0.04, b × d = 0.06, c × d = 0.04, a × b × c = 0.06, a × b × d = 0.08 a × c × d = 0.06, b × c × d = 0.08, a × b × c × d = 0.12									

Explanation see Table 2

biology were most probably related to fungicide persistence in the soil and their degradability (Chen and Edwards 2001).

Sukul (2006) also reported that the activity of soil enzymes was considerably affected by the time of fungicide action. In a 60-day laboratory experiment, metalaxyl (an acylanilide fungicide) at first increased the activity of all soil enzymes tested, i.e. dehydrogenases, phosphatase, arylsulfatase and β-glucosidase, but then caused a decrease in

Table 6. Activity of alkaline phosphatase (mmol PNP/kg dm/h) in the soil contaminated with fungicides

Fungicide dose[k]	Soil cropped with spring barley					Uncropped soil				
	time of fungicide action				*r*	time of fungicide action				*r*
	14	28	42	56		14	28	42	56	
Swing Top 183 SC										
0	1.32	1.84	2.06	1.95	0.81*	1.35	1.19	1.42	1.38	0.42
1	1.48	2.07	2.32	2.22	0.86**	1.31	1.25	1.63	1.62	0.82**
10	1.52	1.54	1.95	1.85	0.42	1.31	1.25	1.42	1.42	0.74*
100	1.36	1.24	1.66	1.60	0.73*	1.28	1.18	1.39	1.42	0.71*
r	−0.23	−0.86**	−0.82**	−0.82**		−0.36	−0.54	−0.47	−0.26	
Unix 75 WG										
0	1.32	1.84	2.06	1.95	0.81**	1.35	1.19	1.42	1.38	0.42
1	1.36	1.75	2.26	2.09	0.86**	1.36	1.22	1.44	1.45	0.59
10	1.37	1.62	2.24	2.17	0.92**	1.33	1.22	1.45	1.48	0.74*
100	1.37	1.62	2.03	1.97	0.90**	1.13	1.18	1.44	1.43	0.91**
r	0.95**	−0.57	−0.63	−0.34		−0.98**	−0.54	0.04	−0.02	
$LSD_{P<0.01}$	a = 0.02, b = 0.03, c = 0.02, d = 0.03, a × b = 0.04, a × c = 0.03, a × d = 0.04 b × c = 0.04, b × d = 0.06, c × d = 0.04, a × b × c = 0.06, a × b × d = 0.09 a × c × d = 0.06, b × c × d = 0.09, a × b × c × d = 0.13									

Explanation see Table 2

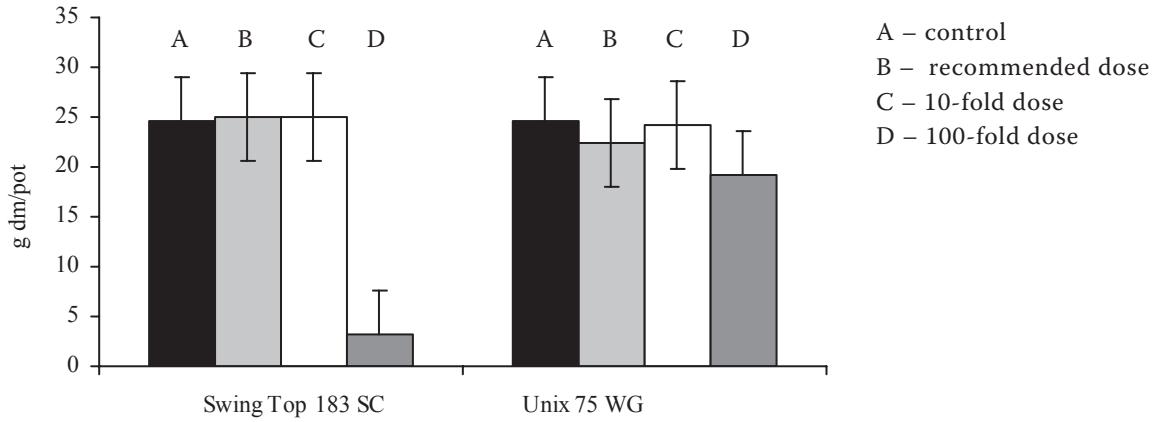

Figure 1. Yield of spring barley in g dm/pot

enzymatic activity. Only urease activity remained at a low level over the entire experimental period.

The yield of spring barley cv. Start was dependent on the type and dose of fungicide (Figure 1). The fungicides applied at recommended doses and at a 10-fold dose had no significant effect on spring barley yields. Only the highest (100-fold) dose caused a significant yield decrease, i.e. by 88% (Swing Top 183 SC) and 22% (Unix 75 WG).

Other authors studied the effects of fungicides containing such active substances as strobilurins (Ray et al. 2004, Ruske et al. 2004, Pasquer et al. 2005), epoxiconazole (Benton and Cobb 1995, Ray et al. 2004) and cyprodinil (Ray et al. 2004), and found that all of them positively affected crop yields when applied at recommended doses. This meets the expectations of both producers and farmers. However, despite yield increment, the fungicides caused anatomical and physiological changes in crops. Strobilurin fungicides (Pasquer et al. 2005) and epoxiconazole (Benton and Cobb 1995) increased the chlorophyll content of plants. According to Ruske et al. (2004), the application of strobilurin fungicides reduced the concentration of proteins and sulfur in winter wheat grain (cv. Malacca). Benton and Cobb (1995) demonstrated that epoxiconazole reduced the length of *Galium aparine* shoots, but had no significant effect on a decrease in dry matter content. This was possible due to increased thickness of spongy parenchyma and elongation of palisade parenchyma cells of the leaves.

In addition, spring barley yield was positively correlated with the activity of the enzymes tested, i.e. dehydrogenases, urease, alkaline phosphatase and acid phosphatase – the coefficients of correlation at $P > 0.01$ were as follows: 0.49**, 0.31**, 0.38** and 0.36**, respectively. This is consistent with the findings of other authors (Myśków 1981, Martyniuk et al. 1998). Myśków (1981) observed a positive correlation between dehydrogenases activity and the yields of maize and oats, whereas Martyniuk et al. (1998) between acid phosphatase activity and barley yield.

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Corresponding author:

Dr. Ewa Jastrzębska, University of Warmia and Mazury in Olsztyn, Department of Microbiology, 10-727 Olsztyn, Plac Łódzki 3, Poland
e-mail: ewa.jastrzebska@uwm.edu.pl