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INFLUENCE OF POWDERY MILDEW ON CEREAL APHIDS IN WHEAT

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Abstract: The leaves of wheat infected by powdery mildew *Erisiphe graminis* var. *tritici* manifested repellent action against cereal aphids *Rhopalosiphum padi* and *Sitobion avenae*. Food (leaves) for aphids enriched in chemical components present in diseased leaves caused weaker reproduction of *S. avenae* and mortality of *R. padi*. It could be caused by post-infection increase of the level of monophenols and flavanoids in the leaves of wheat infected by powdery mildew (*E. graminis* var. *tritici*). These compounds increasing IAA-oxidase activity in plants can have a harmful influence on the cereal aphids.

Key words: cereal aphids, powdery mildew, phenolic compounds

INTRODUCTION

In breeding experiment of cereal aphids (*S. avenae, R. padi*) conducted in climatic chamber we observed that aphids did not settle or reproduced in smaller extent on the seedlings of wheat infected with powdery mildew *E. graminis*. Ruszkowska (personal communication) observed similar phenomenon. Therefore we decided to find the reason and character of this phenomenon.

It could have been expected that an infection of wheat plants with powdery mildew results in the accumulation of mycotoxins or stimulation a biosynthesis of metabolites toxic for host plant as well as for the future parasites, e.g. the aphids. The second possibility seems to be more probable. Although there is no information about relations: wheat – powdery mildew, based on the literature concerning different pathogens it may be assumed that such activity may be shown by many phenolic compounds. There may be compounds that are synthesised *de novo* and fulfilling the role of phytoalexins or there may be phenylpropanoid acids that can occur also in healthy plant at low concentrations (Matern and Grimming 1994; Feuch and Treutter 1989).

Van Emden (1973) indicated secondary plant metabolites as the factors regulating the behaviour of insects. Insects feeding on the plants infected by microorganisms find among nutrients many new compounds that may unfavourably affect their development or in contrary, improve their life conditions.

Stark et al. (2001) observed increased habituation and reproduction of spider-mites on the leaves of apple and cherry infected by powdery mildew. Hence, besides studies on the behaviour of aphids feeding on the wheat infected by powdery mildew we tested the level of phenolic compounds in plants infected by pathogen in different extent.

MATERIALS AND METHODS

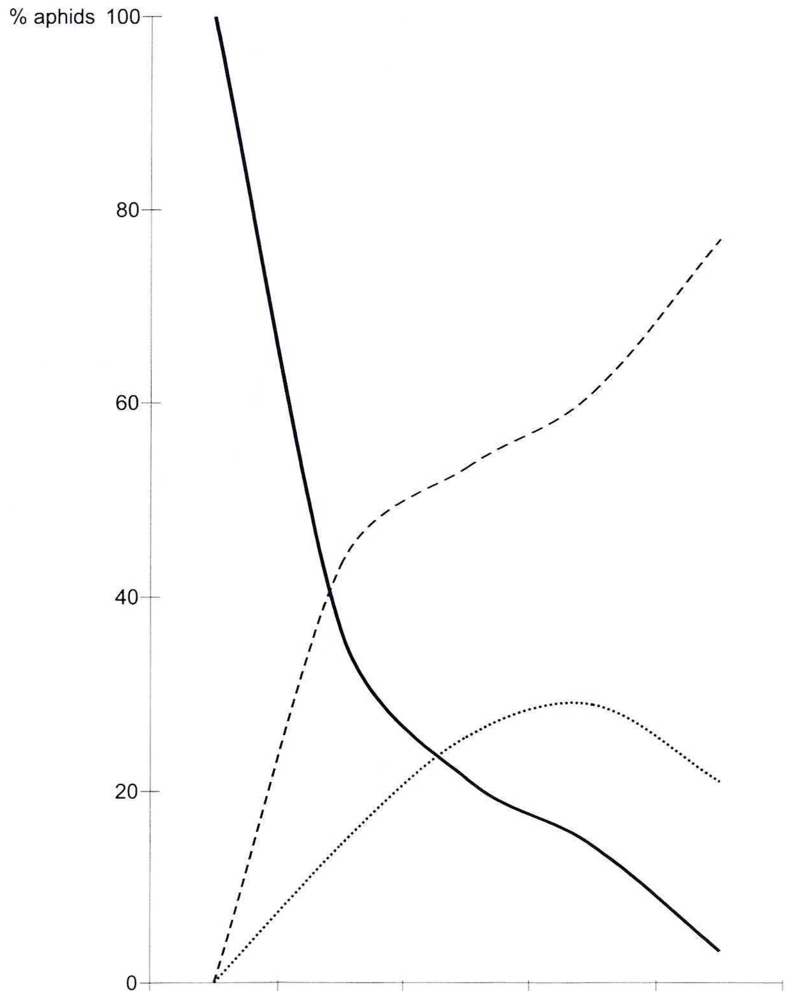
Ethanol extracts were prepared by homogenisation of wheat seedlings (at the stage of three leaves) – healthy and infected with powdery mildew. One ml of extract was equal to 0.5 g of fresh weight of leaves. In aim to prepare water extract 5 ml of ethanol extract were evaporated at 40–50°C and the residue was diluted in 50 ml of water.

In the first stage of studies the preference tests were performed. In these tests the leaves of wheat seedlings were soaked for 10 min. in water extracts of wheat – the healthy one and the plant infected with powdery mildew. Then the leaves were placed in Petri dishes and next 10 aphids of species *S. avenae* (wingless females) were placed in the central point. Petri dishes were kept in a climatic chamber in complete range of light. The second type of tests was conducted as parallel in darkness. Every hour the choice of feeding site was observed and the aphids on the individual seedlings were counted. The tests were performed in four replications and in three series.

The second type of experiments concerned breeding of aphids on the wheat seedlings taking up extracts prepared from healthy wheat and the wheat infected with powdery mildew. The extracts were poured to glass vials; the wheat seedlings were put in them for four hours then the aphids were placed on the seedlings (two aphids per seedlings in three replicates). The experiment was performed in three series and three times. The influence of substances occurred in extracts on development and feeding of the cereal aphids was tested in climatic chamber (16/8 hours light/dark, temp. 22/18°C, humidity 75–80%). The experiment was continued for 5 days.

In the healthy and infected wheat leaves the content of total phenols was estimated with Folin reagent according to colorimetric method (Swain and Hillis 1959). Monophenols were separated from polyphenols on aluminium oxide column (Zuker and Ahrens 1958). Similar analyses of phenols were conducted on wheat seedlings incubated with extracts of healthy and infected wheat for 24, 48 and 72 hours.

The described above ethanol extracts were separated on TLC plates covered with silica gel by developing in mixture: ethyl acetate + formic acid + acetic acid + water (100:11:11:27). The compounds present in extracts were visualised in UV light following treatment of chromatographic plate with 1% methanol solution of diphenylboric acid (1) or Benedict reagent (2), or in visible light after treatment with 1% vanillin solution in sulphuric acid (3).

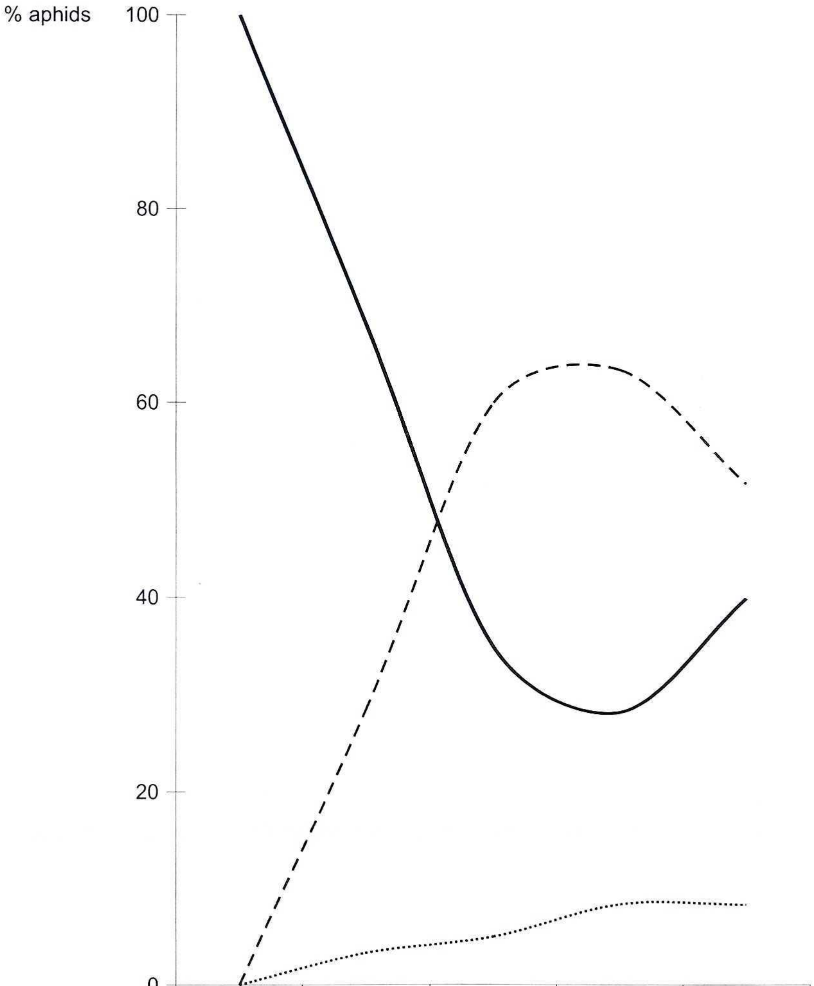


hours	0	1	2	3	4
– – – wetted leaves	0	43.3	53.3	61.1	77
·········· extract-covered leaves	0	14.4	25.6	29	21
—— nonsettling aphids	100	36.7	21.4	14.4	3.3

Fig. 1. Nutritive preference wingless females of *S. avenae*. Wheat leaves covered with extract obtained from plants infected with powdery mildew and control leaves covered with water (test in light)

RESULTS AND DISSCUSION

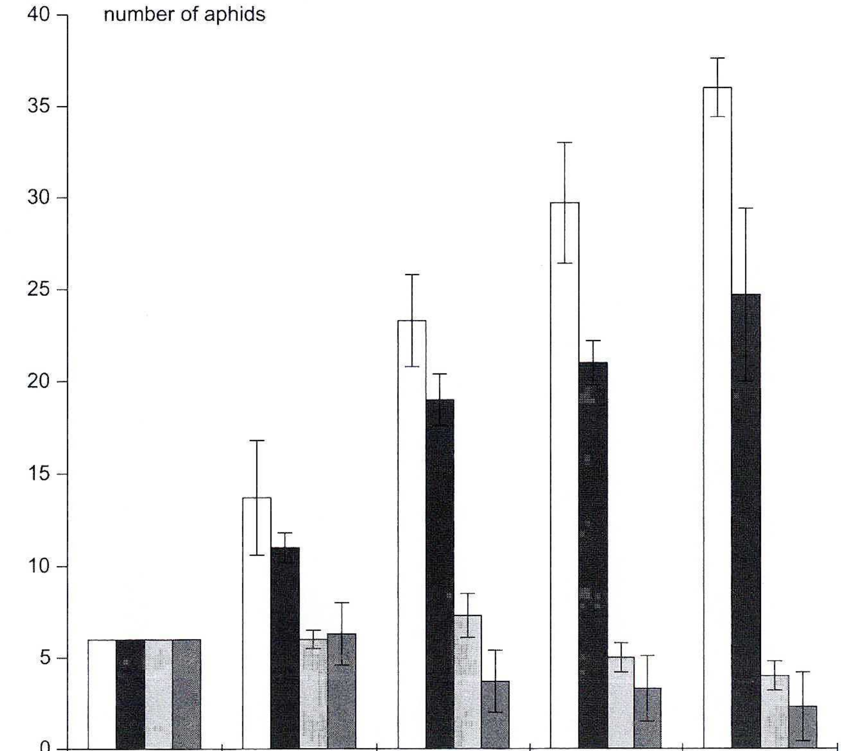
The first experiment concerned the nutritive preference and was performed with wingless females of *S. avenae* both in light and darkness (Figs 1, 2). In both cases, behaviour of aphids was similar, i.e. aphids distinctly avoided leaves covered with



hours	0	1	2	3	4
- - - wetted leaves	0	28.3	60	63.4	51.7
········· extract-covered leaves	0	3.3	5	8.4	8.4
—— nonsettling aphids	100	68.3	35	28.3	40

Fig. 2. Nutritive preference wingless females of *S. avenae*. Wheat leaves covered with extract obtained from plant infected with powdery mildew and control leaves covered with water (test in dark)

extract obtained from plants infected with powdery mildew. This repellent action was stronger in darkness. During 4-hours test, undecided aphids placed earlier in the centre of Petri dishes gradually settled the leaves soaked in water. In the figures 3–5, the results of reproduction of aphids on the wheat leaves nourished with extract from healthy (extract 1) and severe infected leaves (extract 2) are presented. Control plants were fed only with water. These experiments could be conducted for only 5 days since substances present in extracts of diseased leaves caused strong phytotoxic effects. The exchange of nourishment would be difficult and could be harmful to aphids and could cause change of their behaviour.

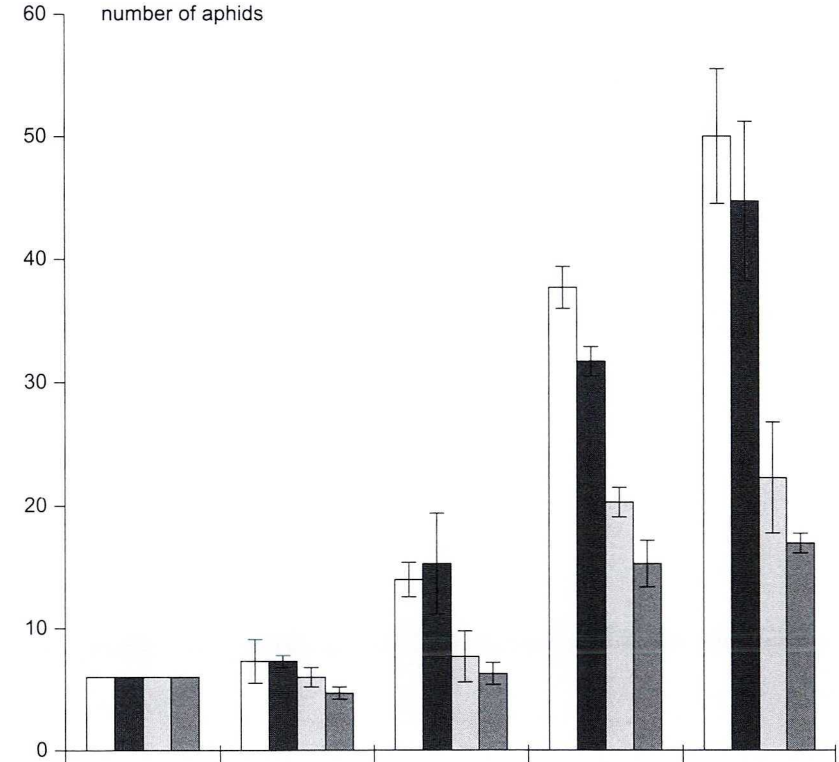


days	1	2	3	4	5
water	6	13.7	23.3	29.7	36
extract 1	6	11	19	21	24.7
extract 2	6	6	7.3	5	4
extract 3	6	6.3	3.7	3.3	2.3

Fig. 3. Reproduction of wingless females of *R. padi* on wheat leaves nourished with solution of extract from healthy (1), weak infected (2) and severe infected (3) wheat leaves

Two-tested aphid species reacted in different way to the post-infection compounds present in the extract of leaves. In case of *R. padi*, aphids were dying with no offspring left. Even the extracts of healthy leaves had negative effect on the development on this species, while it had no effect on *S. avenae* (Fig. 3). The leaves extracts did not kill *S. avenae*, but cause a 50–60% decrease of their reproduction (Figs 4, 5). It seems that winged females of this species were less sensitive than the wingless one.

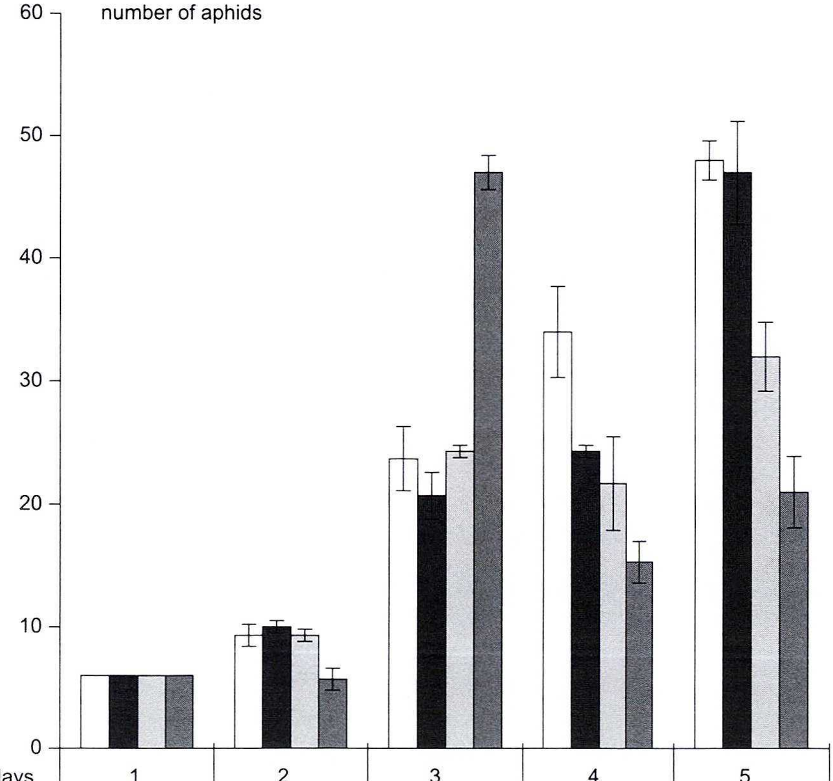
It is necessary to ask a question whether negative action of the extracts from diseased leaves, especially on the *R. padi*, is a result of toxic activity of post-infection metabolites contained in extracts or whether the aphids die or reproduce weaker in



days	1	2	3	4	5
water	6	7.3	14	37.7	50
extract 1	6	7.3	15.3	31.7	44.7
extract 2	6	6	7.7	20.3	22.3
extract 3	6	4.7	6.3	15.3	17

Fig 4. Reproduction of wingless females of *S. avenae* on wheat leaves nourished with solution of extract from healthy (1) weak infected (2) and severe infected (3) wheat leaves

effect of starvation as result of repellent action of the extracts. The second possibility seems to be less probable since close contact of aphids with extract apply to hydroponics was little in comparison with direct one when the leaves were covered with extract through submersion in its solution. Therefore our studies were focused on the search of chemical agent responsible for the behaviour of the cereal aphids on the wheat infected with powdery mildew. We had directed our attention to plant phenols, many of which demonstrate high biological activity (Feucht and Treutter 1989). In result of strong infection by powdery mildew the level of total phenols increased by 50%. However, the part of total phenols was monophenols which amount was doubled while the amount of polyphenols did not change (Tab.



days	1	2	3	4	5
water	6	9.3	23.7	34	48
extract 1	6	10	20.7	24.3	47
extract 2	6	9.3	24.3	21.7	32
extract 3	6	5.7	47	15.3	21

Fig. 5. Reproduction of winged females of S. *avenae* on wheat leaves nourished with solution of extract from healthy (1) weak infected (2) and severe infected (3) wheat leaves

1). These type of changes lead to pathological changes in plant. Monophenols as the activators of monooxidases will accelerate senescing processes including necrosis of the leaf tissue. It can be expected that this type of phenols, especially phenylpropanoid acids, will also limit development of aphids by stimulation of IAA oxidase and hence the catabolism of indoleacetic acid in the plant as well in the aphid. Bur (1985) demonstrated that IAA is necessary for the development of aphids. It was confirmed later by Giebel and Woda-Leśniewska (2001) by application in studies of aphids the inhibitors and activators of IAA oxïdase. Presented above action of monophenols both on the plant and insect organism can be reversed by polyphenols which usually synergies IAA action (Hemberg 1951, Henderson and Nitsch 1962). In our case, in healthy plants and in plants weakly infected with powdery mildew the activity of phenols is "equalized" and the ratio of mono to poly-phenols is 1.1 –1.4 (Tab. 1). If biological activity of phenols observed here is high and the tested extracts significantly limited development of aphids, a question arises whether the phenols from extract of infected plants reached the leaves immersed in its solution. The results in table 2 indicate a 7–14% increase of the phenol level in comparison to control plants, i.e., "fed" with water only for 72 hours. Maybe the reason of it was a low concentration of extracts in water solutions. However, increase of the extract concentration caused already after several hours yellowing and then necrosis of the leaf tissues what made impossible for aphids to feed.

On the other hand, considering obtained biological effect (Figs 3, 4, 5) it can be concluded that the extract concentration was sufficient. Maybe some phenols, contained in extract, had a high abilities of direct action on the aphids or indirect, by *de novo* synthesis other biologically active compounds.

Analysis of extracts by a TLC technique allowed us for approximate determination of quality and the level of phenols (Fig. 6, Tab. 3). Phenolic acids in healthy and diseased plants were detected on the same level. These two groups of plants were

Table 1. Phenolic content in wheat seedlings infected with powdery mildew

Seedlings	Phenols μg/g f.w.			Monophenols/Polyphenols
	total	mono-	poly-	
Healthy	1960	1140	800	1.4
Weak infected (5–10% of leaf area)	1960	1040	920	1.1
Severe infected (70–80% of leaf area)	2840	2040	800	2.6

Table 2. Phenolic content in wheat seedlings fed in water solutions of ethanol extracts from healthy and powdery mildew infected leaves

Treatment	Total phenols μg/g f.w.		
	24 hs	48 hs	72 hs
Extract of healthy leaves	1840	1860	2320
Extract of severe infected leaves	2060	2100	2200
Control (water)	1880	2001	2040

Table 3. Phenolic compounds from ethanol extracts of wheat leaves revealed by TLC technique (see Fig. 6, the chromatograms of these compounds)

Detector	Compound's R_f	Phenolics level	
		in healthy plant	in diseased plant
(A) Diphenylboric acid	0.14	0	++
	0.27	trace	++
	0.34	+	++
	0.38	0	+++
	0.41	+	+++
	0.45	0	+++
	0.62	0	+++
(B) Benedict's reagent (copper sulphate and sodium citrate)	0.15	++	0
	0.70	+++	0
(C) Sulphuric acid and methanol 1:1	0.14	+	++
	0.41	trace	+
	0.62	+++	trace
	0.75	0	+
	0.93	+	++
	0.99	+++	+++
Vanillin reagent	0.08	++	++++
	0.32	trace	trace
	0.37	+	trace
	0.71	trace	+
	0.79	++	+

O – absent, Graduation of the level: +, ++, +++, ++++

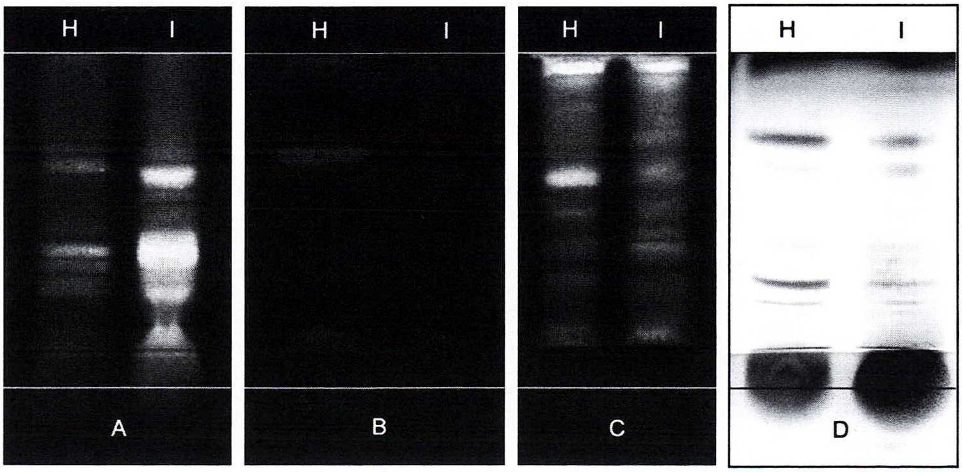


Fig. 6. TLC-chromatograms of ethanolic extracts from healthy (H) and infected (I) with powdery mildew wheat leaves

Detection: Phenylboric acid/polyethyleneglycol-reagent and UV–365 nm (A), Benedict's reagent and UV-365 nm (B), Sulphuric acid and UV–365 nm (C), Vanillin-reagent (D) different in that, that the healthy plants contained more coummarins with o-dihydroxy groups, typical flavanoles and flavones than the healthy plants. However, diseased plants contained a high level of flavanoids, catechine and tannins.

Catechine and other flavanols are able to inactivate the phosphorylation (Paliyath and Poovaiak 1984). Stenlid (1963) found that some flavanoids activate IAA oxidation in plants and can catch free radicals. Therefore, it is not excluded that the flavanols are responsible for aphicidal action of powdery mildew diseased wheat.

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POLISH SUMMARY

ODDZIAŁYWANIE PSZENICY PORAŻONEJ MĄCZNIAKIEM (*ERISIPHE GRAMINIS* VAR. *TRITICI*) NA MSZYCE ZBOŻOWE

Liście pszenicy zainfekowane mączniakiem *Erisiphe graminis* var. *tritici* działały repelentnie w odniesieniu do mszyc zbożowych *Rhopalosiphum padi* i *Sitobion avenae*. Liście traktowane ekstraktami z zainfekowanych pszenic obniżały reprodukcję *S. avenae* oraz powodowały wzrost śmiertelności *R. padi*. Mogło to być wynikiem postinfekcyjnego wzrostu poziomu monofenoli oraz flawanoidów w liściach pszenicy porażonej mączniakiem. Być może związki te przejawiają aficydalne działanie przez aktywowanie IAA-oksydazy i zmianę metabolizmu nie tylko rośliny-gospodarza ale również mszycy.