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THE ROLE OF SOME SECONDARY METABOLITES IN THE HEALTH STATUS OF SWEET PEPPER (*Capsicum annuum* L.) GROWN IN THE FIELD

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Abstract. Secondary metabolites play an important role in plant protection mechanisms. Presented studies relate to the significant role of some phenolic compounds in health status of sweet pepper plants. Eight cultivars of sweet pepper ('Barbórka', 'Caryca F_1', 'Mercedes', 'Ożarowska', 'Podstolina', 'Roberta F_1', 'Robertina') were investigated in 2007–2009. Flavonoids and phenol acids content in leaves, stems, and roots of sweet pepper plants were analyzed. Flavonoids and phenol acids content was studied using isocratic HPLC method. Disease index of tested cultivars was evaluated in the field. The leaves, stems and roots of pepper were analyzed in the laboratory. The fungi most frequently isolated from pepper were *Alternaria alternata*, *Fusarium* spp., *Botrytis cinerea*, and *Sclerotinia sclerotiorum*. The highest content of flavonoids and phenol acids was in the leaves of pepper. Flavonoids content in plant parts was positively correlated with the intensity of colonization by pathogenic fungi. Sweet pepper cultivars 'Caryca F_1' and 'Roberta F_1' were characterised by low content flavonoids in the tested plant parts and they were colonized by the low number of pathogenic fungi. The cultivars most frequently colonized by pathogenic fungi such as 'Barbórka', 'Podstolina', 'Robertina', had a high content of flavonoids in the tested plant parts. There was no correlation between the content of phenol acids and health status of pepper cultivars.

Key words: health status of sweet pepper, cultivars, flavonoids, phenolic acids, fungi

INTRODUCTION

Cultivated plants are attacked by agrophages. Contrary to the natural plant communities, an environment potentially susceptible to pathogens' attack is created in agrocenoses. Pathogenic microorganisms colonizing plants synthesize compounds called elicitors. These substances are activated in plants at the moment of attack. One of the examples of acquired resistance is the accumulation of various biologically active compounds, the so-called organic toxins. There are saponins, tannins and phenols [Talcott

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and Howard 1999, Kozłowska and Konieczny 2003]. Synthesis of structural compounds created in the site of infection is a natural mechanism leading to the physical cut-off of the tissues attacked by the pathogen. Compounds which are toxic to microorganisms and which are synthesized by a plant in response to the pathogen's attack are important elements of the strategy of the attacked organism [Wittstock and Gershenzon 2002]. Bravo [1998] divided them into 10 classes, such as phenolic acids, flavonoids, tannins etc. Phenolic compounds are plant secondary metabolites that constitute one of the most common and widespread groups of substances in plants. Biochemical activity of flavonoids and their derivatives is determined by the presence and mutual orientation of active groups, mainly hydroxyl, methyl and glycoside ones. These compounds are characterized by a high capacity to absorb UV radiation, which is one of the functions ascribed to them is plant protection against the harmful effect of this radiation. Plants need phenolic compounds for pigmentation, growth, reproduction and for many other functions. Flavonoids and phenol acids play a potential role in disease resistance [Hahlbrock and Scheel 1989, Harborne 1999]. Literature reports the effect of phenol compounds on some pathogenic fungi (*Colletotrichum circinans*, *Verticillium albo-atrum*, *Phytophthora infestans*, *Botrytis cinerea*, *Monilinia fructicola*) [Wilson and Wisniewski 1989].

The aim of the paper is to estimate the content of some secondary metabolites, namely flavonoids and phenol acids in the plants of sweet pepper cultivated in the field and their effect on the health status of some cultivars of pepper.

MATERIAL AND METHODS

Plant material. The experiments were conducted in 2007–2009 in Zezulin near Lublin (Lubelskie province). The object of study was eight cultivars of sweet pepper plants (*Capsicum annuum* L.): 'Barbórka', 'Caryca F_1', 'Mercedes', 'Ożarowska', 'Podstolina', 'Roberta F_1', 'Robertina', 'Rumba F_1'. Mineral fertilization was used in accordance to fertilization recommendations for Solanaceae plants on based on the earlier soil analyses with subsequent ploughing. The pepper plants were planted in the field in mid May in 35 × 67 spacing. The experimental combination consisted of 60 plants of each cultivar (10 plants in 6 replicates). Two sweet pepper raws were planted around the experimental plants, and they were not used for the study. Pesticides were not applied and weeds were removed manually. Weather parameters, measured at Meteorological Station Felin (Lublin), were achieved from the Laboratory of Agrometeorology, University of Life Sciences in Lublin.

Biochemical analysis of plants. Leaves, stems and roots of the investigated pepper cultivars were analyzed in Central Laboratory of Agroecology, University of Life Sciences in Lublin. At the full fruiting stage (beginning of September), six plants were randomly collected from each cultivar. Plants were dried in shade and air and then ground. The flavonoids and total phenolic acids contents were determined.

Flavonoid analysis. Determination of flavonoids content (flavonoles converted for quercetine) was performed by means of spectrophotometry [Polish Pharmacopoeia VI 2002].

Analysis of phenolic compounds of o-dihydroxophenol type. Determination of phenolic compounds (with conversion to caffeic acid) was performed by spectrophotometric method according to modified Singelton and Rossi [1965] method.

The analysis of variance and Tukey's HSD test at 5% significance level (SAS Version 9.1, SAS Inst., Cary, N.C., U.S.A.) was applied for data analysis.

Study in the field. During full fruiting (first decade of September), the incidence and the extent of infection symptoms caused by *Alternaria* spp. on the leaves of sweet pepper was estimated. Percentage of infected leaves and disease index of leaves for sixty plants for each cultivars of pepper were estimated using a 5-degree scale (0–5°) (tab. 1). The disease index of leaves (DI) was calculated for each cultivar using Townsend and Heuberger formula [Wenzel 1948]:

$$DI(\%) = \frac{\sum_{0}^{i}(n \cdot v)}{i \cdot N} \cdot 100$$

where:

n – number of plant in the highest degree of disease,
v – degree of disease,
i – the high degree of disease scale,
N – total number of tested plants.

Table 1. Scale used for evaluation of alternariosis on the leaves of sweet pepper

Degree	Description
0°	lack of disease symptoms
1°	small, yellow spots on the leaves up to 25% surface of leaves
2°	yellowish leaves and small necrotic spots on the leaves up to 50% surface of leaves
3°	necrotic spots on the leaves from 50% to 75% surface of leaves
4°	wide necrotic spots, yellowish leaves more than 75% surface of leaves

Data were analyzed by analysis of variance (Tukey's HSD test) at 5% significance level using the SAS statistical system (SAS Version 9.1, SAS Inst., Cary, N.C., U.S.A.).

Mycological analysis of plants. During full fruiting (first decade of September), 6 randomly selected sweet pepper plants were sampled from each plot. The pepper plants (leaves, stems and roots) were analyzed in laboratory. Plant material was precleaned, rinsed with running water for 20 minutes and then surface disinfected with 50% ethyl alcohol and 0.1% sublimate for 1 minute. Disinfected plant material was rinsed 3 times in distilled water. Next, 3 mm fragments were placed on mineral medium in Petri dishes as described by Jamiołkowska [2007]. For each experimental treatment 10 dishes with plant material, 10 plant fragments of fruit per each dish, were prepared and incubated in the thermostat at 20–22ºC for 7 days in darkness. The obtained fungal colonies were

transferred to potato dextrose medium (PDA, Difco) and identified to the species with the available monographs.

RESULTS

Biochemical analysis of plants. Contents of flavonoids and phenolic acids in pepper plants are presented in tables 3 and 4. Contents of flavonoids varied and they depended on weather conditions and on the researched plant part. The highest concentration of flavonoids occurred in aboveground parts of pepper plant. 'Robertina', 'Barbórka', 'Mercedes', 'Ożarowska', 'Podstolina' cultivars were characterised by the highest mean content of flavonoids in leaves. 'Caryca F_1', 'Roberta F_1' and 'Rumba F_1' had the lowest concentration of flavonoids in leaves (fig. 1). Concentration of flavonoids in leaves was statistically higher in leaves than in stems (tab. 3). There were no differences in the content of flavonoids in stems between the cultivars. The content of flavonoids in roots was low but the differences between cultivars are statistically significant. 'Caryca F_1' had the lowest mean concentration of flavonoids in roots (tab. 3, fig. 1).

Table 2. Average monthly temperature (°C) and rainfall in months (mm)

Year	Average monthly temperature (°C)					Sum of rainfall in months (mm)				
	V	VI	VII	VIII	IX	V	VI	VII	VIII	IX
2007	15.0	18.1	19.2	18.4	13.0	81.5	87.8	87.0	37.6	129.8
2008	12.8	17.7	18.3	19.3	12.6	101.6	25.9	77.1	45.0	102.2
2009	13.6	16.4	19.9	19.0	15.3	71.1	125.5	57.1	54.7	21.0
Multiannual mean for 1951–2005	13.0	16.2	17.8	17.1	12.6	57.7	65.7	83.5	68.6	51.6

The highest concentration of phenolic acids occurred in leaves and was similar in all cultivars (tab. 4, fig. 2). The statistically significant differences between cultivars in the content of phenol acids in stems were noticed. The highest mean content of phenolic acids was noticed for 'Barbórka', and the lowest one for 'Roberta F_1', 'Mercedes', 'Ożarowska', 'Postolina', 'Rumba F_1' (fig. 2). The biggest differences between cultivars were noticed in the phenolic acids content in roots. The highest content of phenol acids was noticed in roots of 'Rumba F_1' and 'Barbórka', and the lowest one in 'Roberta F_1', 'Caryca F_1' and 'Mercedes' roots (tab. 4, fig. 2).

Study in the field. At the plant growth stage of full fruiting necrotic spots were observed on the leaves of pepper (phot. 1). Weather conditions in 2007–2009 were very favourable for the development of alternariosis (tab. 2). The highest mean disease index (ID) was noticed for 'Podstolina' (46.1%), and the lowest one for 'Robertina' (29.8%) (tab. 5). Disease index for the tested cultivars varied statistically and its values depended on cultivars and weather conditions (tab. 5).

Table 3. The content of flavonoids converted to quercetine (mg 100 g d.w.) in the leaves, stems and roots of sweet pepper

Plant part	Cultivar	Year		
		2007	2008	2009
Leaf	B	819.037 F*	718.12 C*	290.00 D*
	CF1	381.950 H	314.75 E	566.67 AB
	M	952.397 B	988.27 B	353.33 CD
	O	863.897 E	1125.89 A	373.67 BCD
	P	906.147 C	436.83 D	533.33 ABC
	RF1	734.467 G	357.46 E	314.33 D
	RB	986.070 A	1068.73 A	656.67 A
	RUF1	885.030 D	213.07 F	203.67 D
	LSD (0.05)	5.8186	64.872	203.36
Stem	B	115.923 A*	17.367 D*	49.333 A*
	CF1	35.153 E	61.750 BC	8.967 C
	M	68.847 D	66.593 BC	13.333 C
	O	100.417 B	96.723 A	16.000 BC
	P	72.507 D	58.620 C	27.333 B
	RF1	87.700 C	10.687 D	43.800 A
	RB	73.893 D	109.927 A	56.667 A
	RUF1	113.330 A	76.440 B	12.000 C
	LSD (0.05)	8.9087	16.039	13.138
Root	B	21.823 B*	21.627 B*	12.200 A*
	CF1	2.383 F	4.820 C	3.300 A
	M	9.433 D	11.833 BC	4.000 A
	O	5.146 E	7.957 C	6.433 A
	P	21.433 B	7.650 C	8.667 A
	RF1	18.536 C	5.473 C	1.573 A
	RB	8.213 D	7.767 C	8.700 A
	RUF1	27.653 A	58.467 A	2.267 A
	LSD (0.05)	1.4161	11.542	12.438

B – Barbórka, CF1 – Caryca F_1, M – Mercedes, O – Ożarowska, P – Podstolina, RF1 – Roberta F_1, RB –Robertina, RUF1 – Rumba F_1, * values marked with the same letters (A, B, C...) within columns do not significantly differ at 5% error (Tukey's HSD test)

Mycological analysis of plant. Mycological analysis in 2007–2009 resulted in 6064 isolates of fungi. The predominating species were *Alternaria alternata*, *Fusarium* spp., *Botrytis cinerea* and *Sclerotinia sclerotiorum* (tab. 6–8). The fungi isolated from leaves were *A. alternata, Fusarium* spp. and *B. cinerea*. These fungi were isolated mainly from

Table 4. The content of free phenolic acids converted to caffeic acid (mg 100 g d.w.) in the leaves, stems and roots of sweet pepper

Plant part	Cultivar	Year		
		2007	2008	2009
Leaf	B	896.00 AB*	896.00 B*	1512.00 C*
	CF1	979.43 A	979.43 A	1468.67 D
	M	410.82 D	591.73 E	1232.66 E
	O	485.19 CD	485.20 F	1594.00 B
	P	736.10 ABC	736.10 C	1488.67 D
	RF1	658.10 BCD	658.13 D	1097.33 F
	RB	659.30 BCD	659.30 D	1745.00 A
	RUF1	926.47 AB	926.47 B	918.00 G
	LSD (0.05)	306.08	41.746	21.836
Stem	B	785.95 A*	785. 933 A*	328.78 A*
	CF1	484.16 B	516.533 B	293.02 A
	M	183.33 DE	183.300 EF	339.70 A
	O	239.30 CD	239.300 D	265.13 A
	P	200.85 DE	200.833 E	284.74 A
	RF1	167.50 E	167.500 F	368.31 A
	RB	294.08 C	294.067 C	403.56 A
	RUF1	273.18 C	273.167 C	254.91 A
	LSD (0.05)	60.045	22.495	247.43
Root	B	873.24 A*	328.78 A*	248.73 C*
	CF1	206.87 CD	293.02 A	247.62 C
	M	131.62 D	339.70 A	337.64 BC
	O	323.21 C	265.13 A	337.01 BC
	P	511.17 B	284.74 A	525.66 A
	RF1	72.01 D	368.31 A	441.50 AB
	RB	614.12 B	403.56 A	395.86 AB
	RUF1	896.84 A	254.91 A	354.33 BC
	LSD (0.05)	155.27	247.43	132.82

B – Barbórka, CF1 – Caryca F_1, M – Mercedes, O – Ożarowska, P – Podstolina, RF1 – Roberta F_1, RB – Robertina, RUF1 – Rumba F, * as in table 3

'Podstolina' and 'Mercedes' (fig. 3). Pathogenic fungi obtained from stems were *Fusarium* spp., *B. cinerea*, *S. sclerotiorum* and they were frequently isolated from 'Podstolina' and 'Robertina' (fig. 4). Those cultivars were characterized by the highest content of flavonoids in their leaves and stems (fig. 1). The lowest number of fungal colonies was isolated from 'Caryca F_1' who has low content of flavonoids and phenol acids

Table 5. Percentage of infected leaves and disease index of leaves of sweet pepper cultivated in the field

Cultivar	Percentage of infected leaves (%)			Disease index (%)			Mean of ID (%) 2007–2009
	2007	2008	2009	2007	2008	2009	
Barbórka	100.0 A*	100.0 A*	100.0 A*	18.8 E*	47.4 C*	46.1 D*	37.4
Caryca F_1	98.1 BC	100.0 A	100.0 A	17.1 F	47.3 C	52.5 B	38.9
Mercedes	96.0 C	98.2 AB	100.0 A	13.5 G	43.9 D	44.4 E	33.9
Ożarowska	100.0 A	96.5 B	100.0 A	29.2 AB	37.9 E	49.7 C	38.9
Postolina	99.1 AB	100.0 A	100.0 A	29.5 A	55.1 A	53.6 A	46.1
Roberta F_1	98.2 BC	96.6 B	100.0 A	28.3 B	52.6 B	43.5 F	41.5
Robertina	100.0 A	98.2 AB	100.0 A	22.0 D	54.7 A	34.5 G	29.8
Rumba F_1	98.1 BC	96.5 B	100.0 A	26.4 C	31.9 F	50.0 C	36.1
Mean	98.68	98.25	100.0	23.10	46.35	46.78	37.8
LSD (0.05)	1.6331	2.298	0.000	0.9358	1.352	0.5654	

* as in table 3

Table 6. Fungi colonizing leaves of sweet pepper cultivated in the field

Year	Fungus species	Cultivar								Total (%)
		B	CF1	M	O	P	RF1	RB	RUF1	
2007	*Alternaria alternata* (Fr.) Keiss.	94	91	100	75	52	98	100	93	703 (93.1)
	Epicoccum nigrum Link	–	2	–	–	–	–	–	–	2 (0.3)
	Fusarium avenaceum (Corda ex Fries) Sacc.	4	6	–	4	–	–	–	2	16 (2,1)
	Fusarium equiseti (Corda) Sacc	–	–	–	19	11	–	–	4	34 (4.5)
	total	98	99	100	98	63	98	100	99	755 (100.0)
2008	*Alternaria alternata* (Fr.) Keiss.	87	91	94	94	96	96	97	94	749 (95.7)
	Epicoccum nigrum Link	5	5	–	3	4	–	3	–	20 (2.5)
	Fusarium avenaceum (Corda ex Fries) Sacc.	3	–	–	–	–	–	–	–	3 (0.4)
	Fusarium equiseti (Corda) Sacc.	–	–	–	–	–	3	–	–	3 (0.4)
	Penicillium expansum Link ex S.F. Gray	2	–	–	–	–	–	–	–	2 (0.2)
	Trichoderma hamatum (Bonord) Bain	–	–	6	–	–	–	–	–	6 (0.8)
	total	97	96	100	97	100	99	100	94	783 (100.0)
2009	*Alternaria alternata* (Fr.) Keiss.	91	100	55	89	45	96	91	95	662 (83.7)
	Botrytis cinerea Pers. ex Fries	–	–	45	–	50	3	–	–	98 (12.4)
	Fusarium avenaceum (Corda ex Fries) Sacc.	–	–	–	–	–	–	4	–	4 (0.5)
	Fusarium culmorum (Smith) Sacc.	3	–	–	–	5	–	5	3	16 (2.0)
	Fusarium equiseti (Corda) Sacc.	5	–	–	–	–	–	–	–	5 (0.6)
	Fusarium oxysporum Schlecht.	–	–	–	6	–	–	–	–	6 (0.8)
	total	99	100	100	95	100	99	100	98	791 (100.0)

B – Barbórka, CF1 – Caryca F_1, M – Mercedes, O – Ożarowska, P – Podstolina, RF1 – Roberta F_1, RB – Robertina, RUF1 – Rumba F_1

Table 7. Fungi colonizing stems of sweet pepper cultivated in the field

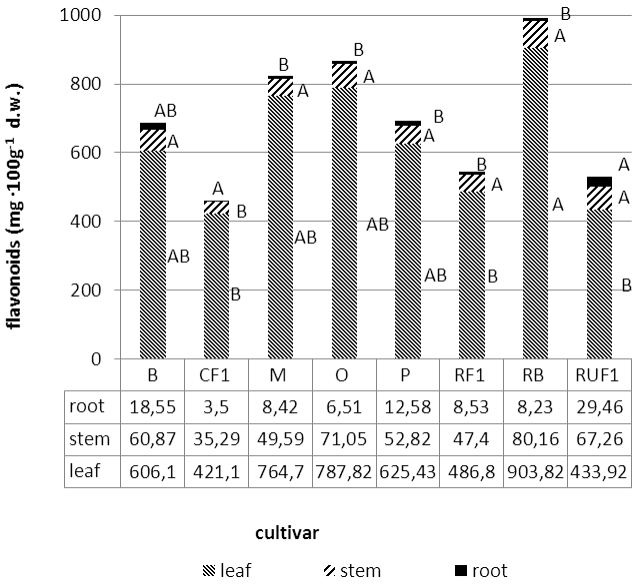
Year	Fungus species	Cultivar								Total (%)
		B	CF1	M	O	P	RF1	RB	RUF1	
2007	*Alternaria alternata* (Fr.) Keiss.	55	42	20	36	4	17	73	75	322 (46.3)
	Aureobasidium pullulans (de Bary) Arnaud	–	–	2	–	–	–	–	–	2 (0.3)
	Botrytis cinerea Pers. ex Fries	–	–	–	–	51	–	–	–	51 (7.3)
	Epicoccum nigrum Link	–	2	2	–	–	–	4	–	8 (1.2)
	Fusarium avenaceum (Corda ex Fries) Sacc.	6	12	2	4	–	2	2	–	28 (4.0)
	Fusarium culmorum (Smith) Sacc.	–	–	–	2	1	–	–	5	8 (1.2)
	Fusarium equiseti (Corda) Sacc.	5	6	9	21	3	–	10	17	71 (10.2)
	Fusarium semitectum Berk. et Rav.	–	–	4	–	–	19	–	–	23 (3.3)
	Gliocladium catenulatum Gilman et Abbott	–	–	4	–	–	–	–	–	4 (0.6)
	Mucor hiemalis Wehmer	–	–	2	2	–	–	–	–	4 (0.6)
	Penicillium expansum Link ex S.F. Gray	–	–	3	–	–	–	–	–	3 (0.4)
	Sclerotinia sclerotiorum (Lib.) de By	32	13	31	20	33	8	3	–	140 (20.1)
	Trichoderma hamatum (Bonord) Bain	–	6	–	–	–	7	–	–	13 (1.9)
	Trichoderma harzianum Rifai	–	–	–	–	–	18	–	–	18 (2.6)
	total	98	81	79	85	92	71	92	97	695 (100.0)
2008	*Alternaria alternata* (Fr.) Keiss.	29	39	41	27	21	44	42	27	270 (43.5)
	Alternaria tenuissima	–	–	–	–	7	–	–	–	7 (1.1)
	Aureobasidium pullulans (de Bary) Arnaud	–	11	–	4	–	–	2	4	21 (3.4)
	Botrytis cinerea Pers. ex Fries	–	–	–	–	–	11	–	–	11 (1.8)
	Chaetomium globosum Kunze ex Steud.	–	–	2	–	2	–	–	1	5 (0.8)
	Cladosporium cladospoioides (Fres.) de Vries		–	–	2	1	–	–	–	3 (0.5)
	Epicoccum nigrum Link	4	8	–	2	6	4	8	3	35 (5.6)
	Fusarium avenaceum (Corda ex Fries) Sacc.	6	2	16	17	23	2	13	19	98 (15.8)
	Fusarium culmorum (Smith) Sacc.	5	1	–	–	–	1	1	–	8 (1.3)
	Fusarium equiseti (Corda) Sacc.	3	8	1	8	21	20	4	18	83 (13.4)
	Mucor mucedo Mich. Ex St.-Am.	–	4	–	1	–	–	3	1	9 (1.4)
	Penicillium expansum Link ex S.F. Gray	5	–	1	6	3	3	1	1	20 (3.2)
	Penicillium janthinellum Biourge	–	–	1	–	–	–	–	–	1 (0.2)
	Trichoderma hamatum (Bonord) Bain	22	–	4	–	–	–	–	3	29 (4.7)
	Trichoderma harzianum Rifai	–	9	5	–	–	–	–	–	14 (2.2)
	Micelia sterilia	–	–	–	–	2	4	1	–	7 (1.1)
	total	74	82	71	67	86	89	75	77	621 (100.0)
2009	*Alternaria alternata* (Fr.) Keiss.	35	5	6	24	14	4	54	18	160 (20.4)
	Aureobasidium pullulans (de Bary) Arnaud	16	28	31	27	–	40	21	10	173 (22.2)
	Botrytis cinerea Pers. ex Fries	–	10	49	–	9	3	4	4	79 (10.1)
	Colletotrichum coccodes (Wallr.) Hughes	–	–	–	–	–	–	–	1	1 (0.1)
	Epicoccum nigrum Link	6	4	2	6	6	1	–	6	31 (4.0)
	Fusarium avenaceum (Corda ex Fries) Sacc.	6	1	6	–	20	2	6	45	86 (11.0)
	Fusarium culmorum (Smith) Sacc.	22	1	–	–	7	1	13	2	46 (5.9)
	Fusarium equiseti (Corda) Sacc.	2	–	–	–	–	–	–	–	2 (0.3)
	Fusarium oxysporum Schlecht.	1	1	–	2	–	–	1	–	5 (0.6)
	Humicola fuscoatra Traaen	8	6	–	10	32	–	–	–	56 (7.2)
	Humicola grisea Traaen	–	19	–	21	–	–	–	–	40 (5.1)
	Trichoderma hamatum (Bonord) Bain	–	–	1	–	–	–	1	–	2 (0.3)
	Trichoderma harzianum Rifai	–	13	5	6	10	49	–	12	95 (12.2)
	Micelia sterilia	–	2	–	1	2	–	–	–	5 (0.6)
	total	96	90	100	97	100	100	100	98	781 (100.0)

B, CF1, M, O, P, RF1, RB, RUF1 – as in table 6

Table 8. Fungi colonizing roots of sweet pepper cultivated in the field

Year	Fungus species	Cultivar								Total (%)
		B	CF1	M	O	P	RF1	RB	RUF1	
2007	*Alternaria alternata* (Fr.) Keiss.	–	–	–	–	2	7	–	–	9 (1.7)
	Colletotrichum coccodes (Wallr.) Hughes	–	–	–	2	–	1	1	–	4 (0.8)
	Fusarium avenaceum (Corda ex Fries) Sacc.	–	5	–	–	–	–	1	–	6 (1.1)
	Fusarium culmorum (Smith) Sacc.	8	3	6	4	3	–	6	2	32 (6.0)
	Fusarium equiseti (Corda) Sacc.	10	4	26	1	4	–	3	–	48 (9.1)
	Fusarium oxysporum Schlecht.	10	15	16	2	–	10	1	11	65 (12.3)
	Fusarium semitectum Berk. et Rav.	–	5	–	22	–	18	–	4	49 (9.2)
	Fusarium solani (Mart.) Sacc.	–	3	–	–	13	–	6	–	22 (4.2)
	Humicola fuscoatra Traaen	2	2	–	1	1	–	4	–	10 (1.9)
	Mortierella isabellina Oud.	–	–	–	–	–	–	1	–	1 (0.2)
	Mucor hiemalis Wehmer	3	7	8	1	–	–	–	12	31 (5.8)
	Mucor mucedo Mich. Ex St.-Am.	–	2	9	–	3	4	–	1	19 (3.6)
	Penicillium chrysogenum Thom	–	1	–	–	–	1	–	–	2 (0.4)
	Sclerotinia sclerotiorum (Lib.) de By	38	5	6	25	43	–	–	45	162 (30.6)
	Trichoderma hamatui (Bonord) Bain	–	–	–	–	5	4	–	5	14 (2.6)
	Trichoderma harzianum Rifai	–	–	–	–	3	3	50	–	56 (10.5)
	total	71	52	71	58	77	48	73	80	530 (100.0)
2008	*Alternaria alternata* (Fr.) Keiss.	–	–	5	–	–	–	–	4	9 (1.9)
	Aureobasidium pullulans (de Bary) Arnaud	–	–	–	5	–	–	–	–	5 (1.1)
	Colletotrichum coccodes (Wallr.) Hughes	2	1	–	11	–	–	–	10	24 (5.2)
	Epicoccum nigrum Link	–	–	–	3	–	–	–	1	4 (0.9)
	Fusarium avenaceum (Corda ex Fries) Sacc.	6	6	4	6	6	10	9	5	52 (11.2)
	Fusarium equiseti (Corda) Sacc.	12	1	15	1	6	1	–	–	36 (7.7)
	Fusarium oxysporum Schlecht.	7	4	15	22	11	10	4	8	81 (17.4)
	Fusarium solani (Mart.) Sacc.	–	–	1	–	–	–	–	–	1 (0.2)
	Gliocladium catenulatum Gilman et Abbott	2	23	–	–	2	1	–	2	30 (6.5)
	Mucor mucedo Mich. Ex St.-Am.	–	–	–	–	–	–	–	6	6 (1.3)
	Papularia irregularis Hotson	–	–	–	–	4	–	–	–	4 (0.8)
	Penicillium cyclopium Westl.	–	–	–	–	–	–	4	–	4 (0.8)
	Penicillium janthinellum Biourge	–	–	1	–	–	–	1	2	4 (0.8)
	Rhizoctonia solani Kühn	4	–	5	4	–	28	6	–	47 (10.1)
	Trichoderma hamatumi (Bonord) Bain	4	9	4	19	24	–	–	3	63 (13.5)
	Trichoderma harzianum Rifai	–	23	9	–	6	18	22	4	82 (17.6)
	Trichoderma koningii Oud.	–	–	–	–	–	–	6	–	6 (1.3)
	Micelia sterilia	–	2	–	3	–	1	2	–	8 (1.7)
	total	37	69	59	74	59	69	54	45	466 (100.00)
2009	*Alternaria alternata* (Fr.) Keiss.	1	1	–	–	6	–	–	10	18 (2.8)
	Aureobasidium pullulans (de Bary) Arnaud	15	–	38	–	–	14	–	–	67 (10.4)
	Botrytis cinerea Pers. ex Fries	–	7	–	6	–	–	–	–	13 (2.0)
	Colletotrichum coccodes (Wallr.) Hughes	–	–	–	20	1	–	–	30	51 (7.9)
	Gliocladium catenulatum Gilman et Abbott	–	–	–	–	3	–	2	3	8 (1.3)
	Fusarium avenaceum (Corda ex Fries) Sacc.	3	1	7	6	–	1	1	–	19 (3.0)
	Fusarium culmorum (Smith) Sacc.	–	4	–	–	3	–	–	2	9 (1.4)
	Fusarium equiseti (Corda) Sacc.	–	–	–	–	1	–	–	–	1 (0.2)
	Fusarium oxysporum Schlecht.	12	8	15	5	3	12	29	10	94 (14.6)
	Humicola brevis Gilman et Abbott	3	62	–	32	–	–	19	2	118 (18.4)
	Mucor hiemalis Wehmer	–	–	–	–	15	–	–	12	27 (4.2)
	Penicillium cyclopium Westl.	–	3	1	3	1	1	–	1	10 (1.6)
	Penicillium expansum Link ex S.F. Gray	–	–	–	–	–	1	–	1	2 (0.3)
	Rhizoctonia solani Kühn	–	–	–	4	–	–	–	–	4 (0.6)
	Trichoderma harzianum Rifai	29	8	10	9	54	47	33	2	192 (29.9)
	Trichoderma koningii Oud.	–	–	–	–	–	9	–	–	9 (1.4)
	total	63	94	71	85	87	85	84	73	642 (100.00)

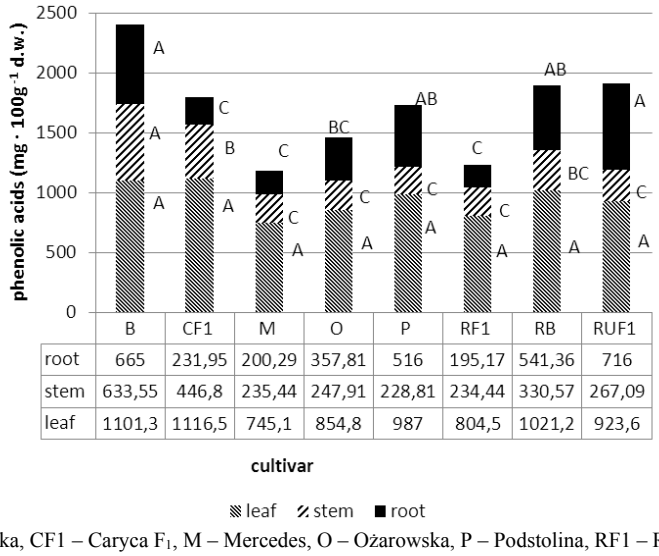
B, CF1, M, O, P, RF1, RB, RUF1 – as in table 6



	B	CF1	M	O	P	RF1	RB	RUF1
root	18,55	3,5	8,42	6,51	12,58	8,53	8,23	29,46
stem	60,87	35,29	49,59	71,05	52,82	47,4	80,16	67,26
leaf	606,1	421,1	764,7	787,82	625,43	486,8	903,82	433,92

B – Barbórka, CF1 – Caryca F_1, M – Mercedes, O – Ożarowska, P – Podstolina, RF1 – Roberta F_1, RB – Robertina, RUF1 – Rumba F_1; Tukey's HSD test LSD (0.05) 378.16 (leaf), LSD (0.05) 49.984 (stem), LSD (0.05) 14.907 (root)

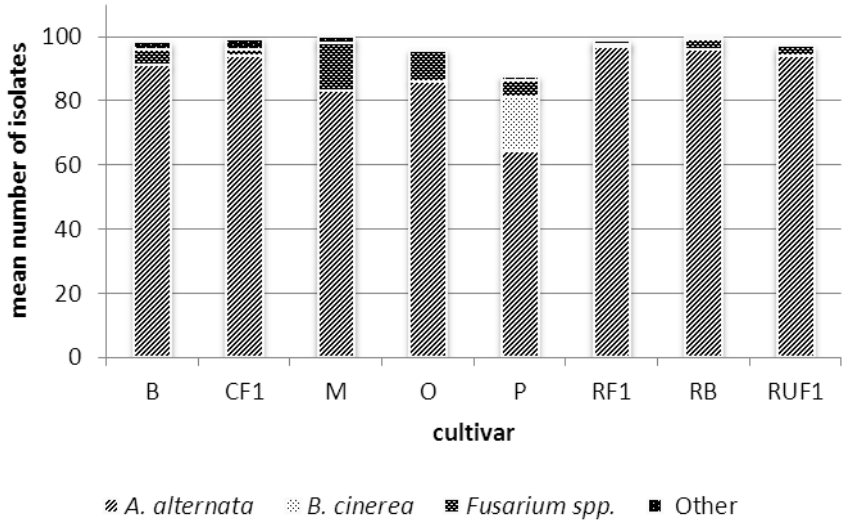
Fig. 1. Mean content of flavonoids converted to quercetine (mg 100 g^{-1} d.w.) in the leaves, stems, roots of pepper in 2007–2009



	B	CF1	M	O	P	RF1	RB	RUF1
root	665	231,95	200,29	357,81	516	195,17	541,36	716
stem	633,55	446,8	235,44	247,91	228,81	234,44	330,57	267,09
leaf	1101,3	1116,5	745,1	854,8	987	804,5	1021,2	923,6

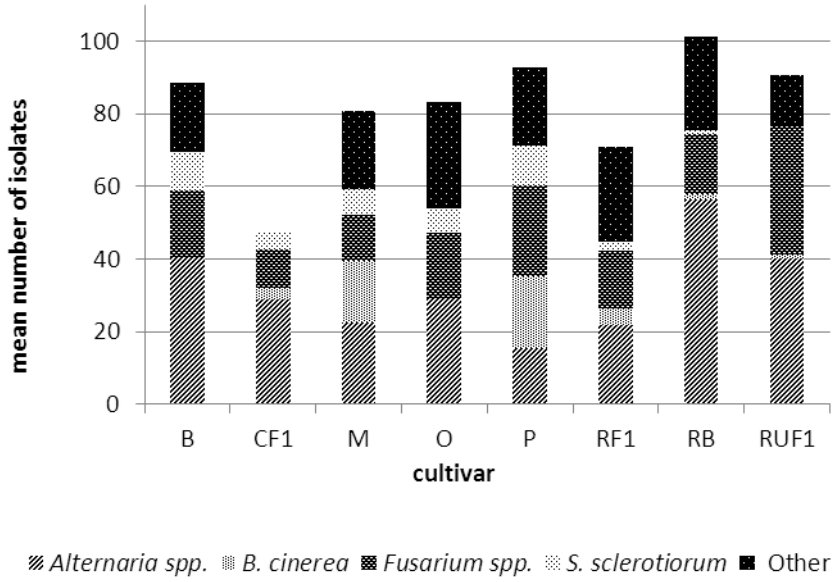
B – Barbórka, CF1 – Caryca F_1, M – Mercedes, O – Ożarowska, P – Podstolina, RF1 – Roberta F_1, RB – Robertina, RUF1 – Rumba F_1; Tukey's HSD test LSD (0.05) 547.12 (leaf), LSD (0.05) 160.69 (stem), LSD (0.05) 257.93 (root)

Fig. 2. Mean content of free phenolic acids converted to caffeic acid (mg 100 g^{-1} d.w.) in the leaves, stems, roots of sweet pepper in 2007–2009



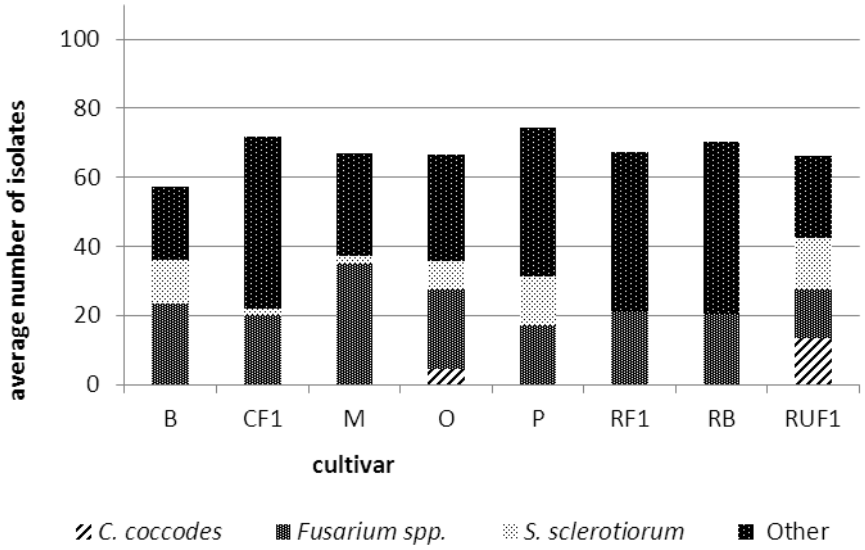
B – Barbórka, CF1 – Caryca F_1, M – Mercedes, O – Ożarowska, P – Podstolina, RF1 – Roberta F_1, RB – Robertina, RUF1 – Rumba F_1

Fig. 3. Mean number of isolates fungi colonizing leaves of sweet pepper in 2007–2009



B – Barbórka, CF1 – Caryca F_1, M – Mercedes, O – Ożarowska, P – Podstolina, RF1 – Roberta F_1, RB – Robertina, RUF1 – Rumba F_1

Fig. 4. Mean number of isolates fungi colonizing stems of sweet pepper in 2007–2009



B – Barbórka, CF1 – Caryca F_1, M – Mercedes, O – Ożarowska, P – Podstolina, RF1 – Roberta F_1, RB – Robertina, RUF1 – Rumba F_1

Fig. 5. Mean number of isolates fungi colonizing roots of sweet pepper in 2007–2009

Phot. 1. Necrotic spots on the leaves of sweet pepper cultivated in the field (phot. by A. Jamiołkowska)

(figs 1–4). *Fusarium* spp., *S. sclerotiorum* i *Colletotrichum coccodes* were frequently isolated from roots of sweet pepper (tab. 8). The lowest number of colonies of pathogenic fungi was isolated from 'Caryca F_1' and 'Roberta F_1' and 'Robertina' roots (fig. 5).

DISCUSSION

Secondary metabolites play an important role in the plant-pathogen interaction [Hahlbrock and Scheel 1989, Candela et al. 1995, Eftekhari et al. 2012]. The present study shows that the content of phenolic compounds in the studied parts of pepper varied. Ruiz and Romero [2001] explain that their content in plants is determined by biotic and abiotic factors. This fact explains the differences in the content of this compounds in different years of the research. One may suppose that the content of flavonoids and phenol acids depended on weather conditions and the analyzed part of the plant. The greatest content of flavonoids was found in the leaves. It is connected with the metabolism of those substances which are accumulated mainly in green parts of plant.

The mycological analysis of pepper shows that *Alternaria alternata* was most frequently isolated from the aboveground parts of pepper. Fungus colonizes the plant without any symptoms [Rotem 1994, Jamiołkowska 2009, Jamiołkowska and Buczkowska 2009]. Yet in favourable conditions, *A. alternata* becomes the pathogen causing necrosis of leaves, stems and fruits. The stems and roots of pepper were also colonized by *Fusarium* spp., *S. sclerotiorum* and *B. cinerea*. As Jamiołkowska [2008] reported *Fusarium* spp. is an important pathogen of pepper cultivated in the field, while *F. oxysporum*, *F. avenaceum*, *F. equiseti* and *F. culmorum* are strongly pathogenic to the seedlings of sweet pepper.

The mycological analysis shown that the hybrid cultivars 'Caryca F_1' and 'Roberta F_1', were the healthiest ones. The lowest content of flavonoids and phenol acids was found in those cultivars. On the basis of biochemical and mycological analyses it can be concluded that these cultivars are characterized by high resistance to pathogenic fungi. Small populations of pathogenic fungi isolated from 'Caryca F_1' and 'Roberta F_1' can be the result of their genetic resistance. The lack of interacion between the pathogen and the plant prevents infection and it determines the phenomenon of resistance [Kozłowska and Konieczny 2003]. The plant's resistance to pathogen can also be the result of callose, lignin and suberine barrier, which protects the plant from the pathogen's penetration [Harborne 1980]. The cultivars 'Podstolina', 'Barbórka' and 'Robertina' were the most colonized by pathogenic fungi such as *S. sclerotiorum*, *B. cinerea* and *Fusarium* spp. These cultivars were characterized by a high average content of flavonoids. Secondary metabolites are synthesized at the moment of pathogen attack and they initiate a number of defensive reactions [Harborne 1980, Kozłowska and Konieczny 2003, Lattanzio et al. 2006]. Other authors [Saniewska 2004, Saniewska and Jarecka 2006] found that flavans, flavonoids, and flavanols contained in grapefruit extract (Citrosept preparation) strongly inhibited linear growth of *F. oxysporum*, *A. alternata*, *Botrytis cinerea* and *Rhizoctonia solani* mycelium *in vitro*. *In vitro* studies reveal that phenolic compounds extracted from olive plant (*Olea europea* L.) fruits: tyrosol, catechin and oleuropein showed antifungal activity, thus affecting plant resistance against *Phy-*

tophthora sp. [Del Rio et al. 2003]. The results obtained in the present study are difficult to analyze in reference to all studied cultivars of pepper. However, it can be stated that there is a relation between the content of flavonoids and the number of pathogenic fungi colonizing the plant tissues. 'Caryca F_1' and 'Roberta F_1', which were characterized by the low content of flavonoids in the leaves, stems and roots, were infected by the pathogenic fungi only to a small degree. 'Podstolina', 'Barbórka' and 'Robertina' were the most colonized by the pathogens and they were characterized by the high content of flavonoids. The obtained results show that a diseased plant reacts to the pathogen's attack producing more phenol compounds than the one which is weakly infected or resistant. At the moment of attack, the synthesis of many biologically active compounds takes place in the plant. It is the protection system against penetration by the pathogen [Harborne 1980, Kozłowska and Konieczny 2003, Lattanzio et al. 2006]. This, however, is a complex process which is difficult to explain on the basis of the conducted fragmentary studies. Candela et al. [1995] observed that an increase of the content of phenol acids occurs in the cultivars resistant to *P. capsici* as compared to sensitive cultivars and the high level of phenolic acids in stems of *C. annuum* determines the plant resistance to *P. capsici*. Harborne [1980] reports that phenolic acid is one of the precursors of lignin formation in tissues of plant. The present study does not show this regularity. The content of phenol acids in the plants of sweet pepper varied and it did not depend on the cultivar or the degree of colonization by pathogenic fungi. The accessible data are difficult to interpret in reference to the effect of phenol acids. Defense reactions created in the plant under the effect of the pathogens' attack are very complex and they depend on many factors.

The present study makes it possible to state that a high content of phenolic compounds in the leaves, stems and roots of the tested cultivars of sweet pepper is the plant's response to the pathogen's attack.

CONCLUSIONS

1. Sweet pepper cultivars tested in the experiment were characterized by different content of flavonoids. Higher flavonoids content in plant parts was positively correlated with the intensity of colonization by pathogenic fungi.
2. 'Caryca F_1' and 'Roberta F_1' cultivars had low contents of flavonoids and were slightly colonized by the pathogenic fungi.
3. 'Barbórka', 'Podstolina', 'Robertina' cultivars had high contents of flavonoids and were the most colonized by pathogenic fungi.
4. The content of phenolic acids in the tested cultivars of sweet pepper was very different. The phenolic acids content in plant parts did not influence the health status of the tested sweet pepper cultivars.

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ROLA NIEKTÓRYCH METABOLITÓW WTÓRNYCH W ZDROWOTNOŚCI ROŚLIN PAPRYKI SŁODKIEJ (*Capsicum annuum* L.) UPRAWIANEJ W POLU

Streszczenie. Metabolity wtórne odgrywają ważna rolę w mechanizmie obronnym rośliny. W przedstawionych badaniach wykazano wpływ związków fenolowych na zdrowotność papryki słodkiej. W latach 2007–2009 przeprowadzono analizę zawartości flawonoidów i kwasów fenolowych w liściach, łodygach i korzeniach papryki słodkiej odmian 'Barbórka', 'Caryca F_1', 'Mercedes', 'Ożarowska', 'Podstolina', 'Roberta F_1', 'Robertina', 'Rumba F_1'. Zawartość flawonoidów i kwasów fenolowych była analizowana techniką HPLC w układzie izokratycznym. Przeprowadzono ocenę stopnia porażenia odmian papryki i wykonano analizę mikologiczną roślin. Grzyby najliczniej izolowane z roślin papryki to *Alternaria alternata*, *Fusarium* spp., *Botrytis cinerea* i *Sclerotinia sclerotiorum*. W liściach i łodygach roślin stwierdzono wysokie stężenie flawonoidów i kwasów fenolowych. Zawartość flawonoidów w badanych częściach papryki była pozytywnie skorelowana z zasiedleniem roślin przez grzyby patogeniczne. Odmiany 'Caryca F1' i 'Roberta F1' charakteryzowały się najniższym stężeniem flawonoidów i słabo zasiedlane były przez grzyby chorobotwórcze. Odmiany 'Barbórka', 'Podstolina', 'Robertina' licznie porażane przez grzyby chorobotwórcze miały wysoką zawartość flawonoidów w badanych częściach roślin. Nie stwierdzono zależności pomiędzy zawartością kwasów fenolowych a zdrowotnością badanych odmian papryki słodkiej.

Słowa kluczowe: zdrowotność papryki słodkiej, odmiany, flawonoidy, kwasy fenolowe, grzyby

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