Turkish Journal of Botany

Volume 39 | Number 1 Article 15

1-1-2015

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Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2015) 39: 147-161

doi:10.3906/bot-1403-106

Fungi occurring on the plants of the genus *Amaranthus* L.

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Received: 31.03.2014 • **Accepted:** 24.07.2014 • **Published Online:** 02.01.2015 • **Printed:** 30.01.2015

Abstract: A study of fungi on *Amaranthus* spp. was performed in 2007–2009. The following forms of the genus were taken under consideration: cultivated amaranth (*A. cruentus*) and a wild form (*A. retroflexus*) growing as a weed on amaranth and sugar beet plantations and growing as a ruderal weed. The aim of the work was to determine which fungi communities occur in the phyllosphere, roots, rhizoplane, and rhizosphere of *Amaranthus* spp. To investigate the phyllosphere fungi communities, 5 plants were taken in the seed formation phase. From each plant, 3 healthy, symptomless leaves were taken. In addition, the isolation of fungi communities from the roots, rhizoplane, and rhizosphere was performed in the seed formation phase. Ten plants from each location were taken along the diagonal of the plot. In total, 38 species of fungi were isolated from the phyllosphere of *Amaranthus* spp., and of that number, 30 were collected from *A. cruentus* and 29 from *A. retroflexus*. In total, 29 fungi species were isolated from the roots of all the observed forms of amaranth. From the rhizosphere of all amaranth species tested, 44 fungi species were isolated. The most frequently recorded taxa within the associations of fungi isolated from the phyllosphere were *Cladosporium* spp. (*C. cladosporioides* and *C. herbarum*), *Alternaria alternata*, and *Epicoccum nigrum*. Fungi species of the genera *Penicillium*, *Fusarium*, *Cladosporium*, and *Phoma*, as well as species of *A. alternata*, were isolated in great abundance from the roots and the rhizosphere of the amaranth plants.

Key words: Fungi, *Amaranthus* spp., phyllosphere, rhizoplane, rhizosphere

1. Introduction

In recent years producers and consumers have been interested in new plant species, which are referred to as alternative plants or new crops. Whether they are actually new or just recently rediscovered, these species not only create valuable crude material for a number of industrial branches but they also constitute an important source of renewable energy. In addition, they add to the human menu, making it more diverse (Nalborczyk, 1999).

One of these plants is amaranth (*Amaranthus* L.), which originated in South America (Piesiewicz and Ambroziak, 1995; Nalborczyk, 1999; Tlustoš et al., 2006). The genus *Amaranthus* includes cultivated forms that are grown for seed, vegetable, or animal feed, as well as wild and decorative species. Of all the cultivated forms, the species best known is *Amaranthus cruentus* L. (Nalborczyk, 1999). It has several common names, including blood amaranth, red amaranth, purple amaranth, and Mexican grain amaranth. Apart from this cultivated form, wild species also abound in the fields of Poland, and these are notorious weeds among major crops and are often difficult to control. The most ubiquitous weed among these is the redroot pigweed, *A. retroflexus* L., predominantly infesting root and tuber crops and showing a considerable level of resistance to a number of herbicides (Rahban, 1993). Amateur plant enthusiasts zealously grow decorative forms of amaranth. In Poland, the foxtail amaranth, *A. paniculatus* L., bearing prominent burgundy inflorescence, is commonly grown as well.

The fungal communities of leaves (phyllosphere) and roots (rhizosphere, rhizoplane) may affect the plant's health status and consequently impact the amount and quality of the crop yield. At prevalent atmospheric conditions that are favorable to the pathogens, the resultant disturbance in the equilibrium between epiphytic pathogens and saprotrophic microorganisms can precipitate plant disease. However, the quantitative and species compositions of the mycobiota infesting plant fungal communities are not only dependent on the atmospheric factors, but also on species and localization (Moszczyńska et al., 2013).

The aim of the presented work is to investigate the species composition of the fungi associated with the

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phyllosphere and the roots (rhizoplane, rhizosphere) of different amaranth forms.

2. Materials and methods

The amaranth experiments were carried out in 2007–2009. The materials for investigation were different forms of blood amaranth, *Amaranthus cruentus* (cultivated form), and the redroot pigweed, *A. retroflexus* (wild form). The plant parts inspected for fungi were the stems, leaves, roots, and rhizosphere.

The field experiment with blood amaranth was set at 2 localities: the experimental farm of the Wrocław University of Environmental and Life Sciences, located in Pawłowice, 15 km NE of Wrocław, and a commercial farming enterprise (AgroPol), located at Łosiów near Brzeg, within the Opolskie region, 60 km SE of Wrocław. In both localities the observations incorporated redroot pigweed plants (*A. retroflexus*) infesting the experimental crops of *A. cruentus*. The wild species occurred in all years of the experiment over the entire area of the blood amaranth crop.

In order to describe the fungi associations of the plant phyllosphere, 5 plants of each species were sampled from both fields (Łosiów and Pawłowice) at the seed-setting stage of the crop. Three leaves with no disease symptoms were detached from each plant and placed in paper envelopes that were kept in a mobile refrigerator while being transported to the laboratory. In the laboratory 4 disks of a 0.5-cm^2 surface area were cut from each leaf blade. The disks were placed into 20-mL glass flasks containing 10 mL of sterile water. The flasks were mounted in an automatic shaker and shaken for 10 min (amplitude: 4250 cycles per minute).

From the obtained rinse water, 1 mL of suspension was taken from each flask and poured into a petri dish in 3 replicates. Next, the cooled Martin medium was poured over the dishes with the addition of tetracycline (Martin, 1950). The fungi colonies growing on the petri dishes were split out and grafted onto PDA medium slants in glass probing tubes, from which the fungi were sampled for species identification.

The isolation of the fungi from the plant roots and the rhizoplane and rhizosphere of all the forms of amaranth were carried out at the seed setting stage. The plants taken for analyses originated from the following localities: *A. cruentus* and *A. retroflexus* from Pawłowice and Łosiów. From each site, 10 plants were sampled along the diagonal of the field. Plants were placed into paper bags and kept in a mobile refrigerator for transport to the laboratory. Isolation of fungi was performed according to the rinse method, as described by Mańka (1974). From every collective sample, i.e. every 10 plants per site, 1 g of roots was weighed out and then shaken for 2 min (amplitude: 4250 cycles per minute) in 10 consecutive Erlenmeyer flasks containing 70 mL of sterile water. Additionally, 30 g of sterile silica sand was added to the ninth flask in turn, in order to facilitate the detaching of anatomically bound fungi from the root surface. After the roots were taken from the last Erlenmeyer flask and dried on sterile blotting paper, they were chopped into 5-mm fragments, and these were placed in petri dishes with solidified PDA medium, 6 fragments per dish. In this manner the fungi associated with the internal root tissues could be isolated.

For the isolation of the rhizosphere and rhizoplane fungi (flasks 2 and 4 or flask 9, respectively), 1 mL of suspension was taken from each flask and distributed with a glass spreader over the solidified Martin medium.

After incubation (22 °C, 2–7 days in darkness), the fungal colonies grown on each of the petri dishes (90 mm in diameter) were counted and identified. The specific identification of the sampled fungi was performed using macro- and microscopic observations, namely the morphology of hyphae, conidia, and sporangia of the colonies that had grown on culture media, according to commonly accepted methods used in mycological laboratories. The fungi were identified using diagnostic keys (Raper and Thom, 1949; Raper and Fennell, 1965; Zycha and Siepmann, 1969; Ellis, 1971; Arx, 1974).

In order to estimate the effect of habitat conditions and sampling locality on the variability in abundance of the species of fungi infesting the species of amaranth tested, log-linear analysis and correspondence analysis (CA) were used. In essence, log-linear analysis allows the investigation of the variation of abundance within cross-tabulation tables; it also tests variability and significance of differences in cases in which data from individual observations are missing (zero values) at any level of the experimental treatments to be analyzed. The log-linear analysis used in the present study has been presented according to Goodman (1971): all significant deviations of the observed frequencies from the expected ones found in this analysis indicate an interaction between the analyzed variables. After the logarithmic transformation of the expected frequencies, the model assumes the linear form, which may be represented by the following formula:

$$\mathrm{Ln}\,(E_{ij}) = M + \lambda_i^X + \lambda_j^Y + \lambda_{ij}^{XY},$$

where E_{ij} is the expected frequencies, M is the general mean based on equal frequencies in every cell of the cross-tabulation table, λ_i^X is the effect of the i*th* observation value taken by the variable X, λ_j^Y is the effect of the j*th* observation value taken by the variable Y, and λ_{ij}^{XY} is the effect of the interaction between the i*th* value of variable X and the j*th* value of variable Y.

The log-linear model allows for verification of the null hypothesis, which assumes no interactions between 2 or more of the analyzed variables. It also makes it possible

to assess, after rejecting the insignificant interactions, the extent to which individual factors contribute to the variability of the surveyed population. Chi-square values for the main effects were calculated in order to test the optimal model for analysis of the particular experimental factors. Next, the extended models were tested, including secondary and tertiary interactions among the investigated variables.

Finally, the effects of study year, sampling site, and plant species on the abundance of the investigated species of fungi were determined. For this purpose, CA, also called the optimal scaling method or homogeneity analysis, was applied, as described by Hill (1974). In CA, the distribution in graphs of the points marked as different experimental treatments represents the extent of variation observed in the original, multidimensional space with respect to the abundance of fungi species.

3. Results

3.1. Mycological analysis

A total of 38 species of fungi were isolated from the phyllosphere of *Amaranthus* spp.; 30 were collected from *A. cruentus* (Table 1) and 29 from *A. retroflexus* (Table 2). Regardless of amaranth form, the fungi most frequently

Table 1. Fungi isolated from phyllosphere of *Amaranthus cruentus* (number of isolates).

Species	Pawłowice			Łosiów		
	2007	2008	2009	2007	2008	2009
Alternaria alternata (Fr.) Keissl.	45	59	65	56	79	82
Alternaria botrytis (Preuss) Woudenberg & Crous	1	1	1	1	2	2
Arthrinium phaeospermum (Corda)	1	1	2			
Botrytis cinerea Pers. ex. Fr.	23	37	34	2	3	2
Cladosporium cladosporioides (Fres.) de Vries	30	38	45	34	45	34
Cladosporium herbarum (Pers.) Link ex S.F.G.	56	67	76	45	78	86
Epicoccum nigrum Link	56	95	103	23	27	25
Geotrichum candidum Link	2	3				
Gibberella avenacea R.J. Cook	45	23	21			
Gibberella fujikuroi (Sawada) Wollenw.	1	3	12			
Gibberella intricans Wollenw.	3	2	9	2	7	6
Gibberella pulicaris (Fr.) Sacc.	3	8	12	1		1
Gibberella zeae (Schwein.) Petch	1	1	2			
Gonatobotryum fuscum (Sacc.)	2	4	4			
Microdochium dimerum (Penz.) Arx	4	6	12			
Mucor hiemalis Wehmer	1	1	2			
Penicillium commune Thom.	1	2	6			
Penicillium expansum Link	4	8	16	12	18	12
Penicillium griseofulvum Dierckx	2	3	3	2	6	6
Penicillium notatum Westling	2	1	4			
Penicillium thomii Maire			3			
Penicillium velutinum Westling	3	6	6	1	1	1
Phoma eupyrena Sacc.	2	5	5			
Phoma herbarum Westd.				1	1	3
Phoma levellei Boerema & Bollen	2	5				
Pleospora herbarum (Pers. ex Fr.) Rabenh.					2	3
Talaromyces luteus C.R. Benj.	2	1	3	1	1	1
Talaromyces purpurogenus (Stoll) Samson, Yilmaz, Frisvad & Seifert	23	32	45	12	23	25
Trichoderma hamatum (Bon.) Bain.	2	6	12			
Trichoderma harzianum Rifai				1		1
Colonies of yeasts	87	145	102			
Dark nonsporulating colonies	2	5	4	12	63	54
Gray nonsporulating colonies	1			1	1	22
Total	407	568	609	207	357	366

Table 2. Fungi isolated from phyllosphere of *Amaranthus retroflexus* (number of isolates).

Species	Pawłowice			Łosiów		
	2007	2008	2009	2007	2008	2009
Alternaria alternata (Fr.) Keissl.	34	56	55	33	50	57
Boeremia exigua (Desm.) Aveskamp, Gruyter & Verkley				2	6	6
Botrytis cinerea Pers. ex. Fr.	11	10	12	8	10	12
Cladosporium cladosporioides (Fres.) de Vries	67	96	103	78	85	89
Cladosporium herbarum (Pers.) Link ex S.F.G.	23	43	32	33	32	32
Epicoccum nigrum Link	16	26	17	16	26	20
Fusarium culmorum (W.G. Smith) Sacc.				1		
Fusarium oxysporum Schlecht.			2			
Fusarium sporotrichioides Sherb.		2	2	1	2	
Geotrichum candidum Link	1	1		1	1	7
Gibberella avenacea R.J. Cook	1	1	2	1	1	2
Gibberella intricans Wollenw.	11	17	21	11	13	11
Gibberella pulicaris (Fr.) Sacc.	1	2	2	2	2	1
Microdochium dimerum (Penz.) Arx	1	1	4	1	1	1
Monographella nivalis (Schaffnit) E. Müll.				2		3
Mucor hiemalis Wehmer	2	2	5	1	2	5
Paraphoma fimeti (Brunaud) Gruyter, Aveskamp & Verkley						1
Penicillium commune Thom.			3	2	4	6
Penicillium expansum Link	16	25	34	11	19	17
Penicillium notatum Westling	3	2	2	1	1	1
Penicillium thomii Maire	2	8	8			
Penicillium velutinum Westling	9	12	12	3	6	6
Phoma herbarum Westd.				2		2
Phoma medicaginis Malbr. & Roum.				1	4	3
Stachybotrys chartarum (Ehrenb.) S. Hughes					1	2
Talaromyces purpurogenus (Stoll) Samson, Yilmaz, Frisvad & Seifert	11	14	11	3	7	9
Trichoderma hamatum (Bon.) Bain.				1		
Trichoderma harzianum Rifai	1	1				
Trichoderma viride Pers ex. S.F. Grey	1		1			
Colonies of yeasts	11		12	1	1	2
Dark nonsporulating colonies	2		11	22	29	12
Gray nonsporulating colonies				11	11	7
Total	224	319	351	249	314	314

isolated from the leaf surface was *Cladosporium* spp. Two exceptions were found in *A. cruentus* from Pawłowice. In Pawłowice, the fungi isolated in greatest abundance from *A. cruentus* were *Epicoccum nigrum*, *Botrytis cinerea*, and yeast-like colonies. The fungi of *Cladosporium* spp. were exemplified by *C. cladosporioides* and *C. herbarum*; the first more often infested the leaves of *A. retroflexus* and the second the leaves of blood amaranth. The pathogenic fungi of *Gibberella* spp. (*G. avenacea* and *G. intricans*) were fairly profuse in the phyllosphere of all the amaranth species, yet they were isolated in low numbers from the leaves of *A. cruentus* grown in Łosiów, regardless of study year. *Alternaria alternata* was often isolated from the leaf surface of the investigated plants. The majority of isolates were taken from the amaranth grown in Łosiów, and *Penicillium* spp. were recorded fairly frequently from all the amaranth species. On the cultivated form, *Talaromyces purpurogenus* occurred quite often, while in wild amaranth

species *P. expansum* was most abundant. In general, the lowest number of isolates of *Penicillium* spp. was obtained in 2007 from the wild species *A. retroflexus* in Łosiów.

In total, 29 fungi species were isolated from the roots of both forms of amaranth (Tables 3 and 4). The highest number of fungal colonies were isolated from the roots of *A. cruentus* grown in Pawłowice, and from the remaining species of *Amaranthus* spp., fewer colonies were obtained. From the 2 amaranth species tested, most isolated fungi were from the genera *Penicillium* and *Fusarium*. On the cultivated form, more of these fungi were obtained from the roots of amaranth grown in Pawłowice as compared to Łosiów, regardless of study year. The diminished abundance of colonies of *Fusarium* spp. and *Gibberella* spp. isolated in Łosiów was accompanied by an increase in *Penicillium* spp. colonies. On the other hand, in the root tissue of redroot pigweed (*A. retroflexus*), the percent proportion of *Fusarium* spp., *Gibberella* spp., and *Penicillium* spp. was approximately the same. In general, *F. oxysporum* predominated among the *Fusarium* spp. isolates. In all study years, this species was most frequently isolated from the amaranth grown in Pawłowice. *Penicillium* spp. was represented by 2 species: *P. notatum* and *P. velutinum*. The first was dominant on the roots of the cultivated form from Pawłowice, whereas *P. velutinum* was obtained from all investigated amaranth forms and its incidence on all of them was similar. Apart from the species listed so far, a number of other taxa were isolated, including *A. alternata*, *Phoma* spp., and *Cladosporium* spp. From the rhizosphere of all amaranth species tested, 44 fungi species were isolated (Tables 5 and 6). The species richness of the fungal associations was always higher in the rhizosphere compared to the rhizoplane. Among the cultivated and wild amaranth, the highest number of fungi was always obtained from the blood amaranth grown in Pawłowice. The wild amaranth form did not vary significantly with

Table 3. Fungi isolated from roots of *Amaranthus cruentus* (number of isolates).

Species	Pawłowice			Łosiów		
	2007	2008	2009	2007	2008	2009
Alternaria alternata (Fr.) Keissl.	2	2	2	1		
Arthrinium phaeospermum (Corda)	1		1			
Aspergillus brasiliensis Varga, Frisvad & Samson				1		1
Cladosporium herbarum (Pers.: Fr.) Link						1
Fusarium oxysporum Schlecht.	34	51	67	5	2	4
Fusarium poae (Peck) Wollenw.	1		1	2	2	5
Fusarium sporotrichioides Sherb.						1
Gibberella avenacea R.J. Cook	4	5	2	2	1	1
Gibberella intricans Wollenw.	1			3	3	3
Microdochium dimerum (Penz.) Arx	1	1				
Monographella nivalis (Schaffnit) E. Müll.				3	3	3
Penicillium claviforme Bainer	2	2	2	2	1	2
Penicillium griseofulvum Dierckx	4	6	6	2	2	2
Penicillium notatum Westling	10	15	26	12	14	11
Penicillium thomii Maire	8	12	12			
Penicillium velutinum Westling	7	12	17	3	11	17
Rhizopus stolonifer (Ehrenb.) Vuill.	1	2	7	3		
Talaromyces luteus C.R. Benj.				2	1	2
Talaromyces purpurogenus (Stoll) Samson, Yilmaz, Frisvad & Seifert	3	6	6	3	4	6
Trichoderma aureoviridae Rifai	1	1	2			
Trichoderma hamatum (Bon.) Bain.	2	4	4			
Trichoderma harzianum Rifai				1	2	5
Yeast colonies	1	1	1			
Dark nonsporulating colonies						2
Total	83	120	156	45	46	66

Table 4. Fungi isolated from roots of *Amaranthus retroflexus* (number of isolates).

Species	Pawłowice			Łosiów		
	2007	2008	2009	2007	2008	2009
Alternaria alternata (Fr.) Keissl	2	2	2	1	1	2
Epicoccum nigrum Link	1	1	1			
Fusarium oxysporum Schlecht.	1	1		3	1	2
Gibberella avenacea R.J. Cook	2	2	2	2	2	1
Gibberella intricans Wollenw.	2	1		1	1	2
Gibberella pulicaris (Fr.) Sacc.	2	2	3	1	2	1
Monographella nivalis (Schaffnit) E.Müll.	11	12	17	9	11	12
Mucor hiemalis Wehmer				2		4
Penicillium commune Thom.	2	3	3			
Penicillium griseofulvum Dierckx	3	2	12			
Penicillium notatum Westling	3	3	14	9	11	11
Penicillium velutinum Westling	11	12	12	9	12	11
Phoma eupyrena Sacc.			2			
Stachybotrys chartarum (Ehrenb.) S.Hughes				1		1
Talaromyces purpurogenus (Stoll) Samson, Yilmaz, Frisvad & Seifert	5	7	3	2	3	3
Trichoderma polysporum (Link ex. Pers) Rifai						2
Trichoderma viride Pers ex. S.F. Gray				2		1
Yeast colonies	1		2		2	4
Total	46	48	73	42	46	57

respect to abundance of the fungi species isolated. Although the species list of the fungi found on the roots of different forms of *Amaranthus* spp. was similar, the frequencies of individual fungi species varied across the host plants investigated. In the rhizosphere and the rhizoplane of all the amaranth forms, *Penicillium* spp. predominated. Their proportion, relative to the general number of isolates, amounted to as much as 75%. The exception was again the blood amaranth (*A. cruentus*) grown in Pawłowice, with its *Penicillium* isolates making up about 16% of all the samples across 2008 and 2009. *Penicillium* spp. was mostly seen as 3 species: *P. velutinum*, *P. thomii*, and *P. expansum*. In addition, *Cladosporium* spp., exemplified by *C. cladosporioides* and *C. herbarum*, was isolated in relatively great abundance from the rhizosphere. The greatest abundance of isolates of these taxa was obtained from the rhizoplane of *A. cruentus* in Pawłowice (27%–49% relative to the total number of isolates). On the other hand, the species belonging to *Fusarium* spp. were isolated infrequently from the rhizosphere of *Amaranthus* spp., in particular from *A. retroflexus*. They constituted up to 17% of the total number of colonies obtained in this manner. Moreover, the species of *Fusarium* spp. were isolated only sporadically from the rhizoplane of the amaranth species tested (up to 7% of the isolates). They were most often found on *A. retroflexus*.

A fungi species found fairly often was *A. alternata*. The isolates of this fungus were obtained in the greatest numbers from the rhizosphere of the redroot pigweed (*A. retroflexus*). Taxa belonging to *Trichoderma* spp. and *Phoma* spp. occurred with low frequency but were found in each year of the study.

The yeast-like fungi were also associated with the *Amaranthus* spp. rhizosphere. They made up 57% of all the isolates obtained in 2008 and 2009. In the majority of cases, these organisms were isolated from the amaranth grown in Pawłowice. The species identified abundantly from the rhizosphere of all amaranth forms tested was *Periconia minutissima*, which constituted 23% of the total number of isolates.

3.2. Statistical analysis: phyllosphere

The optimal statistical model of the effect of plant species, study year, and sampling locality on the number of the fungi species isolated from the amaranth phyllosphere was tested using chi-square tests for the main effects and particular interactions of experimental factors. The high values of statistics calculated for the model, which included secondary and tertiary interactions, allowed for the null hypothesis to be rejected at $P < 0.01$. The inclusion of the secondary and tertiary interactions enhances consistency, which was proven by the significant chi-square values. Table 7 shows the main effects, marginal associations, partial

Table 5. Fungi isolated from rhizosphere of *Amaranthus cruentus* (number of isolates).

Species	Pawłowice			Łosiów		
	2007	2008	2009	2007	2008	2009
Alternaria alternata (Fr.) Keissl.	3	2	4	4	5	5
Arthrinium phaeospermum (Corda)	3	2	3			
Aspergillus niger van Tieghem	1	1				
Aureobasidium pullulans (de Bary) Arnaud	2	5	4			
Botrytis cinerea Pers. ex. Fr.	1	1	4			
Choanephora cucurbitarum (Berk. & Ravenel) Thaxt.				1	1	1
Cladosporium cladosporioides (Fres.) de Vries	110	150	109	12	25	33
Cladosporium herbarum (Pers.) Link ex S.F. Gray	33	24	25		2	2
Epicoccum nigrum Link	3	1	2			
Fusarium culmorum (W.G. Smith) Sacc.	1					
Fusarium poae (Peck) Wollenw.	4	7	14			
Gibberella avenacea R.J. Cook		1	1	1	1	2
Gibberella intricans Wollenw.				2	1	3
Gibberella pulicaris (Fr.) Sacc.		1	1			
Humicola grisea Traaen	1	3	2			
Microdochium dimerum (Penz.) Arx	3	5	8			
Mucor hiemalis Wehmer	4	5	11	1	2	4
Monographella nivalis (Schaffnit) E.Müll.	2	1	3			
Penicillium commune Thom.	2				1	1
Penicillium notatum Westling	1					
Talaromyces luteus C.R. Benj.	1	3	4	1	2	2
Talaromyces purpurogenus (Stoll) Samson, Yilmaz, Frisvad and Seifert	2	3	3	1	3	4
Penicillium thomii K.M. Zalessky	69	57	63	52	58	51
Penicillium velutinum Westling	2	7	7	13	22	23
Penicillium expansum Link	2	22	21		23	24
Penicillium griseofulvum Dierckx	2	10	17	1	2	2
Periconia minutissima Corda	45	73	73	63	72	67
Phoma eupyrena Sacc.	2	3	3			
Phoma exigua Desm.	2	2	2			
Phoma fimeti Brun	1					
Phoma medicaginis Malbr. & Roum.				1	4	2
Rhizopus nigricans Ehrenb.	1				1	
Trichoderma aureoviridae Rifai	1	2	2			
Trichoderma hamatum (Bon.) Bain.	1	4	4	1	3	3
Trichoderma harzianum Rifai	1	2	2	1	2	
Trichoderma koningii Oud.				2	1	1
Trichoderma polysporum (Link ex. Pers) Rifai	1	1	2			
Trichoderma viride Pers ex. S.F. Gray	1	1	1			
Yeast colonies	34	267	248	1	4	4
Dark nonsporulating colonies						1
Gray nonsporulating colonies	3	3	3		3	3
Total	345	669	649	158	238	238

Table 6. Fungi isolated from rhizoplane and rhizosphere of *Amaranthus retroflexus* (number of isolates).

Species	Pawłowice			Łosiów		
	2007	2008	2009	2007	2008	2009
Alternaria alternata (Fr.) Keissl.	4	5	9	5	8	8
Botrytis cinerea Pers. ex. Fr.	1					
Choanephora cucurbitarum (Berkeley & Ravenel) Thaxter	1	1	2	1	1	
Cladosporium cladosporioides (Fres.) de Vries	2	5	5	2	4	4
Cladosporium herbarum (Pers.) Link ex S.F. Gray	2	7	5	3	7	7
Epicoccum nigrum Link			1			
Gibberella avenacea R.J. Cook	1	1				
Fusarium culmorum (W.G. Smith) Sacc.		1		1	1	1
Fusarium oxysporum Schlecht.			11			
Fusarium poae (Peck) Wollenw.	4	4	4	1	4	4
Fusarium sporotrichioides Sherb.			2			
Gibberella intricans Wollenw.						7
Gibberella fujikuroi (Sawada) Wollenw.						3
Microdochium dimerum (Penz.) Arx	2	1	5	1	1	1
Mucor hiemalis Wehmer	2	2	5	1	2	11
Penicillium commune Thom.	1	1			1	2
Talaromyces luteus C.R. Benj.	2	2	4	1	1	2
Talaromyces purpurogenus (Stoll) Samson, Yilmaz, Frisvad & Seifert		1	1			
Penicillium thomii K.M. Zalessky	48	46	51	26	70	65
Penicillium velutinum Westling	1	12	12	1	9	8
Penicillium expansum Link	2	24	34	1	9	5
Penicillium griseofulvum Dierckx	2	5	5	1	2	2
Periconia minutissima Corda	52	73	83	22	52	48
Phoma fimeti Brun			2			
Phoma medicaginis Malbr. & Roum.				1	1	1
Rhizopus stolonifer (Ehrenb.) Vuill.			2	1	1	1
Stachybotrys atra Corda						1
Trichoderma aureoviridae Rifai			2			
Trichoderma hamatum (Bon.) Bain.			2			
Trichoderma harzianum Rifai						
Trichoderma koningii Oud.				1	1	2
Trichoderma polysporum (Link ex. Pers) Rifai					1	1
Trichoderma viride Pers ex. S.F. Gray				1		
Yeast colonies			5			5
Dark nonsporulating colonies						
Gray nonsporulating colonies					4	4
Total	127	191	252	71	180	193

Table 7. Phyllosphere: tests of the main effects, marginal associations (mrg. ass.), partial associations (part. ass.), and interaction of the experimental factors.

Effect	Degrees of freedom	Chi-square, part. ass.	P	Chi-square, mrg. ass.	P
Plant species (1)	1	22.14	<0.0001	22.14	<0.0001
Study years (2)	2	87.61	<0.0001	87.61	<0.0001
Sampling locality (3)	1	30.47	<0.0001	30.47	<0.0001
Fungi species (4)	7	1166.72	<0.0001	1166.72	<0.0001
1 × 2	2	1.39	0.4984	1.64	0.4399
1 × 3	1	0.91	0.3402	5.21	0.0224
1 × 4	7	380.73	<0.0001	385.06	<0.0001
2 × 3	2	2.62	0.2703	2.60	0.2723
2 × 4	14	6.87	0.9396	6.88	0.9391
3 × 4	7	105.77	<0.0001	109.84	<0.0001
1 × 2 × 3	2	4.71	0.0950	2.36	0.3069
1 × 2 × 4	14	8.58	0.8571	8,.68	0.8511
1 × 3 × 4	7	79.56	<0.0001	79.02	<0.0001
2 × 3 × 4	14	12.61	0.5576	10.88	0.6952

associations, and interactions of the experimental factors. Significant interactions were found between plant and fungi species and between sampling locality and fungi species, showing the host plant and sampling locality (habitat conditions) effects on the number of fungi species infesting the amaranth forms investigated. The marginal values for the sampled fungi species show the predominance of *Alternaria alternata* and *Cladosporium herbarum* (Table 8). From the leaves of *Amaranthus retroflexus*, on the other hand, *C. cladosporioides* was isolated in abundance. The environmental conditions in Pawłowice favored the greater abundance of the observed fungi species, whereas their incidence on the leaves sampled from Łosiów was lower. *A. retroflexus* showed an increased degree of resistance to the observed fungi species as compared to *A. cruentus*.

The CA supplies further information on the resistance of the observed amaranth species against the leaf-infesting species of fungi. In Figure 1, vectors representing the

Table 8. Phyllosphere: marginal frequencies of the isolated fungi species as dependent on the plant species and on the sampling locality.

Species	*Amaranthus retroflexus*			*Amaranthus cruentus*		
	Pawłowice	Łosiów	Total	Pawłowice	Łosiów	Total
Alternaria alternata	145	140	285	169	217	386
Botrytis cinerea	33	30	63	94	7	101
Cladosporium cladosporioides	266	252	518	113	113	226
Cladosporium herbarum	98	97	195	199	209	408
Epicoccum nigrum	59	62	121	254	75	329
Gibberella intricans	49	35	84	14	15	29
Penicillium expansum	75	47	122	28	42	70
Talaromyces purpurogenus	36	19	55	100	60	160
Total	761	682	1443	971	738	1709

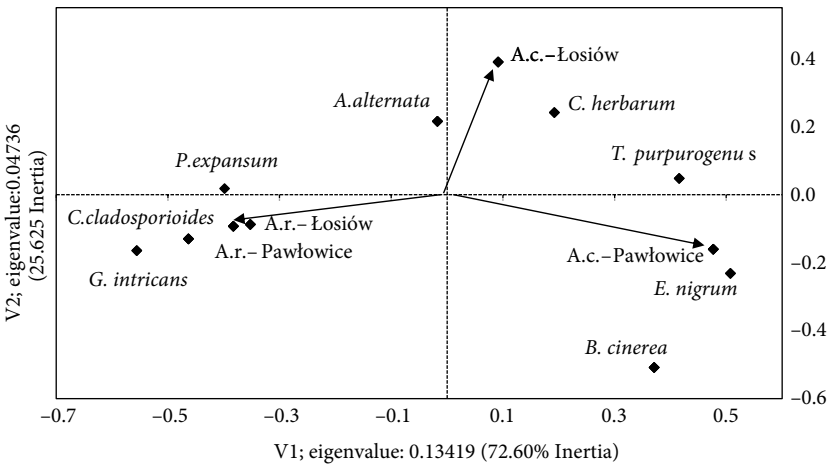

Figure 1. Phyllosphere: biplot of the CA of the isolate variability number, as dependent on environment and species of *Amaranthus*.

variability of *A. cruentus* infestation by the fungi in the 2 sampling localities are found in 2 separate quarters of the graph. Conversely, in the case of *A. retroflexus*, these vectors overlap, indicating the well-balanced response of *A. retroflexus* to the fungi association infesting its leaves, regardless of environmental conditions (Figure 1). The open angle between the vector of *A. retroflexus* and *A. cruentus* confirms the different responses of the 2 plant species to the pathogenic fungi, measured as the number of pathogenic isolates obtained from them. Projections of the points representing the changeable incidence of *A. alternata* and *C. herbarum* onto the vector of *A. cruentus* at the Łosiów sampling locality illustrate the predominance of these 2 fungi species on the leaves of blood amaranth. The same graph validates the prevalence of *Epicoccum nigrum* on *A. cruentus* in the environmental conditions of Pawłowice.

3.3. Statistical analysis: rhizosphere and rhizoplane

Chi-square values for the main effects and for the interactions demonstrate a considerable effect of study year, sampling locality, and species of amaranth on the number of fungal isolates obtained from the plant rhizosphere. The statistics calculated for the model with secondary and tertiary interactions are significant, and therefore the null hypothesis was rejected at $P < 0.01$. The inclusion of the secondary and tertiary interactions in the model enhances its consistency, which was proven by the significant chi-square values (Table 9). The analysis showed great variability in fungi abundance across study years, which confirms the significance of weather conditions for the degree of fungal infestation of the rhizosphere of *A. retroflexus* and *A. cruentus*. Furthermore, a significant variation was found with respect to the number of isolates of particular fungi species, as dependent on the edaphic environment in the respective localities. Based on the results of the log-linear analysis a significant interaction was established between the fungi species and study years and between fungi species and sampling locality, which means that the number of particular isolates was dependent, to a large extent, on environmental conditions. The partial association of the interaction of the amaranth species with sampling locality is insignificant. In the case of no other 2-way interactions, the significant marginal association indicates variation in fungal infestation between the host plant species observed. The predominant species in the rhizosphere, regardless of sampling locality and amaranth species, was *Penicillium velutinum* (Table 10); *C. cladosporioides* was distinct for its greater abundance on the leaves of *A. cruentus* in Pawłowice. The environmental conditions in the latter location favored the greater abundance of the observed fungi species compared to Łosiów. *A. cruentus* showed an increased susceptibility to infestation by the observed fungi as compared to *A. retroflexus*.

The analysis of the CA graph (Figure 2) reveals an enhanced similarity between the 2 sampling localities (Pawłowice and Łosiów) with respect to the number of fungi isolated from the rhizosphere of *A. retroflexus*. The similarity is reflected by the fact that vectors representing the variability of the observed fungi are located within the same quarter of the coordinate system. Conversely, the open angle between the 2 vectors representing *A. cruentus* in Łosiów and Pawłowice points to considerable variation in fungal isolates in the rhizosphere of this plant species. The appreciable dispersion of the points representing the individual species of fungi indicates the changeable effect of *Amaranth* species and environment on the variability of species counts of the investigated fungi. The CA graph supports the notion of the predominance of *C. cladosporioides* in the rhizosphere of *A. cruentus*

Table 9. Rhizosphere: tests of the main effects, the marginal associations (mrg. ass.), the partial associations (part. ass.), and the interaction of experimental factors.

Effect	Degrees of freedom	Chi-square, part. ass.	P	Chi-square, mrg. ass.	P
Plant species (1)	1	198.65	<0.0001	198.65	<0.0001
Study years (2)	2	45.948	<0.0001	45.94	<0.0001
Sampling locality (3)	1	90.42	<0.0001	90.42	<0.0001
Fungi species (4)	5	983.72	<0.0001	983.72	<0.0001
1 × 2	2	15.07	0.0005	15.78	0.0003
1 × 3	1	0.516	0.4723	29.55	<0.0001
1 × 4	5	276.20	<0.0001	301.96	<0.0001
2 × 3	2	11.93	0.0025	13.47	0.00118
2 × 4	10	77.37	<0.0001	75.63	<0.0001
3 × 4	5	164.36	<0.0001	190.95	<0.0001
1 × 2 × 3	2	1.35	0.5066	1.85	0.3946
1 × 2 × 4	10	3.69	0.9600	5.09	0.8844
1 × 3 × 4	5	70.26	<0.0001	73.53	<0.0001
2 × 3 × 4	10	9.66	0.4705	13.19	0.2131

Table 10. Rhizosphere: marginal frequencies of the isolated fungi species as dependent on the plant species and on the sampling locality.

Species	*Amaranthus retroflexus*			*Amaranthus cruentus*		
	Pawłowice	Łosiów	Total	Pawłowice	Łosiów	Total
Alternaria alternata	19	18	37	16	58	74
Cladosporium cladosporioides	12	10	22	369	70	439
Cladosporium herbarum	12	17	29	82	5	87
Penicillium expansum	60	15	75	45	48	93
Penicillium thomii	18	21	39	11	14	25
Penicillium velutinum	145	161	306	189	161	350
Total	266	242	508	712	356	1068

in Pawłowice and the prevalence of *P. velutinum* in the rhizosphere of *A. retroflexus* in Łosiów.

3.4. Statistical analysis: roots

Based on the log-linear analysis of the fungi species found on the roots of *A. cruentus* and *A. retroflexus*, significant differences were recognized in fungi abundance among plant species, study years, and sampling localities (Table 11). The significant interaction between plant species and pathogen species illustrates the variable resistance of *A. cruentus* and *A. retroflexus* to the pathogens present in the observed fungal assemblages. The counts of fungal isolates were also dependent on the environmental conditions at individual sampling localities. The years of the study, as separate sampling seasons, had no effect on the number of fungi species isolated from the plants. Notably less plentiful fungi associations were isolated from the roots of the observed plant species, compared to the rhizosphere or leaves. *A. cruentus* showed a markedly higher susceptibility to the observed pathogenic species than *A. retroflexus* (Tables 11 and 12). Apart from that, the considerable prevalence of *F. oxysporum* on the roots of *A. cruentus* in Pawłowice deserves particular attention.

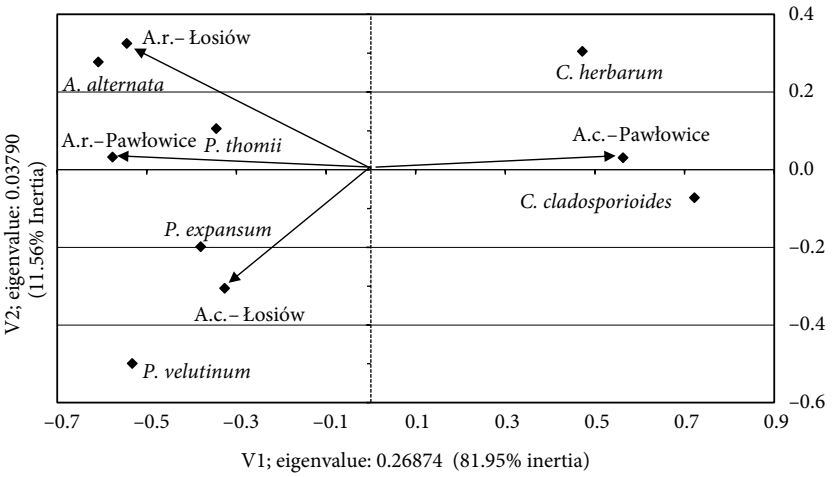

Figure 2. Rhizosphere: biplot of the CA of the isolate variability number, as dependent on environment and species of *Amaranthus.*

Table 11. Roots: tests of the main effects, the marginal associations (mrg. ass.), the partial association (part. ass.), and interaction of the experimental factors.

Effect	Degrees of freedom	Chi-square, part. ass.	P	Chi-square, mrg. ass.	P
Plant species (1)	1	28.92	<0.0001	28.92	<0.0001
Study years (2)	2	24.25	< 0.0001	24.25	<0.0001
Sampling locality (3)	1	43.37	<0.0001	43.37	<0.0001
Fungi species (4)	5	182.87	<0.0001	182.87	<0.0001
1 × 2	2	2.08	0.3521	2.64	0.2660
1 × 3	1	2.40	0.1210	24.44	<0.0001
1 × 4	5	162.08	<0.0001	183.63	<0.0001
2 × 3	2	2.028	0.36	2.20	0.3314
2 × 4	10	3.64	0.96	3.39	0.9706
3 × 4	5	60.05	<0.0001	81.28	<0.0001
1 × 2 × 3	2	0.14	0.92	0.25	0.8825
1 × 2 × 4	10	7.66	0.661893	6.99	0.7260
1 × 3 × 4	5	24.91	0.000145	26.20	<0.0001
2 × 3 × 4	10	11.29	0.334799	11.95	0.2883

The CA graph (Figure 3) indicates that the fungi associations found on *A. retroflexus* are less variable than those of *A. cruentus* plants. The inconstant numerical response of the root-borne fungi in the particular sampling localities may be classified into 3 distinct groups. The first group includes *P. notatum*, *T. purpurogenus*, and *P. velutinum*, which are projected into the first quarter of the graph. Another group consists of *M. nivalis* and *A. alternata*. Finally, the third category contains only *F. oxysporum*, which shows a considerably different kind of variability in the number of isolates obtained from different plant species and different localities.

Table 12. Roots**:** marginal frequencies of the isolated fungi species as dependent on the plant species and on the sampling locality.

Species	*Amaranthus retroflexus*			*Amaranthus cruentus*		
	Pawłowice	Łosiów	Total	Pawłowice	Łosiów	Total
Alternaria alternata	6	4	10	6	3	9
Fusarium oxysporum	3	6	9	152	11	163
Monographella nivalis	40	32	72	3	9	12
Penicillium notatum	20	31	51	51	37	88
Talaromyces purpurogenus	15	8	23	15	13	28
Penicillium velutinum	35	32	67	36	31	67
Total	119	113	232	263	104	367

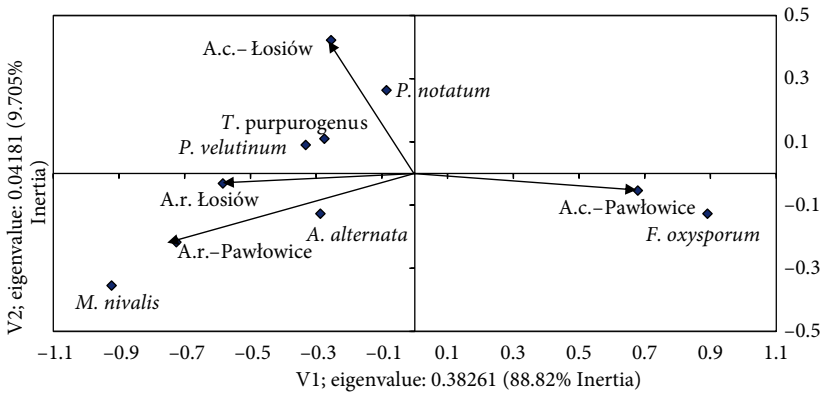

Figure 3. Roots: biplot of the CA of the isolate variability number, as dependent on environment and species of *Amaranthus.*

4. Discussion

The most frequently recorded taxa within the associations of fungi isolated from the phyllosphere are *Cladosporium* spp. (*C. cladosporioides* and *C. herbarum*) and *Alternaria alternata*. Kita (1988) and Kutrzeba (1993) confirmed that these fungi are ubiquitous on the leaf blades of many plant species. In the present study, *C. cladosporioides* was found to infest the leaves of the redroot pigweed (*A. retroflexus*) most frequently, whereas *C. herbarum* most frequently infected leaves of blood amaranth (*A. cruentus*). The greatest number of isolates of *A. alternata* was sampled from the cultivated form grown in Łosiów and the fewest isolates were taken from the decorative form, regardless of study year. Another pathogen, *Botrytis cinerea*, was fairly abundant as well, but occurred exclusively on the leaves of the cultivated form. Although this polyphagous species may infect any plant organ, it is nevertheless most dangerous for the stems, as such infections make the stems die back prematurely (Weber et al., 1992).

The fungi species of the genera *Penicillium*, *Fusarium*, *Cladosporium*, and *Phoma*, as well as *A. alternata*, were isolated in great abundance from the roots and the rhizosphere of the amaranth plants. According to Warcup (1971), these taxa commonly accompany crop plants in a number of different habitats. Some of them, like those belonging to the genus of *Fusarium*, may cause root rot in the cultivated forms of amaranth (Chen and Swart, 2001; Blodgett et al., 2008). However, in the experiment described here, no disease symptoms were found on the plant roots, despite the extensive incidence of these fungi. Among them, the species most frequently isolated was *F. oxysporum*. Although this fungus commonly persists in soil as a saprotrophic (Garret, 1970), it is also known that in favorable conditions it may switch into parasitic mode and become one of the most notorious and widespread pathogens, producing countless biotypes that infect many plant species (Nelson et al., 1981). One of the possible explanations for the nevertheless decent health status of the

amaranth roots observed in our experiment is the incidence of antagonistic organisms (Dorenda, 1982; Weller, 1988; Benhamou et al., 2002). The well-known antagonists are the fungi of the genus *Trichoderma*, characterized by a high growth rate and the ability to produce noteworthy amounts of antibiotic compounds (Salina, 1981; Kredics et al., 2007; Küçük and Kıvanç, 2007; Clarkson et al., 2008). Last but not least, of considerable importance for root health are nonpathogenic strains of *Fusarium* spp., including *F. oxysporum* (Benhamou et al., 2002). It seems likely that this contributed to the results in our experiment.

From both the rhizoplane and the rhizosphere of *Amaranthus* spp., the species of the genus *Penicillium* were isolated in great abundance. They are osmophilic and nitrophilous fungi that frequently occur in nutrient-rich soils (Maciejowska-Pokacka, 1971). Furthermore, the taxa of *Cladosporium* spp. were frequently found in the rhizosphere. These fungi, much like *A. alternata*, usually infest senescing or damaged tissue; therefore, the proportion of the isolates with *Cladosporium* is rather high relative to the total number of isolates collected. The presented results of these experiments concerning fungal associations of the amaranth rhizosphere are consistent with the findings reported by other authors who investigated assemblages of fungi accompanying other crop plant species (Dorenda, 1982; Wagner, 1983; Majchrzak, 1985; Mazur, 1992; Mazur et al., 1992; Pląskowska, 1996). The majority of fungi species isolated from the environment of amaranth vegetation and described in the present work are considered as omnipresent taxa, commonly found in the edaphic environment of crop plants (Henis et al., 1979; Truszkowska et al., 1979).

In conclusion, the log-linear analysis and CA showed that the influence of the atmospheric and edaphic factors on fungi associations infesting the rhizosphere, leaves, and roots of *A. retroflexus* and *A. cruentus* are variable. The fungi assemblages found on the roots are considerably less abundant compared to the phyllosphere of the respective plant species within *Amaranthus* spp. Compared to *Amaranthus cruentus*, a lower number of fungi species were isolated from *A. retroflexus*, regardless of the part of the plant the isolates were taken from. The CA showed that variation between sampling localities in the number of fungi species isolated from *Amaranthus retroflexus* was lower compared to the analogous variation observed in the other species (*A. cruentus*).

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