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PATHOGENS COLONIZING THE ABOVEGROUND PARTS OF POTATO PLANTS IN TREATMENTS WITH FOLIAR FERTILIZATION[1]

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Abstract

The objective of this study was to determine the composition of fungal communities colonizing the leaves and stem base of three potato cultivars. NPK fertilizers were applied to the soil at two levels, and three foliar fertilizers, Basfoliar 12-4-6, ADOB Mn and Solubor DF, were applied alone and in combinations. Yeast-like fungi were most frequently isolated from the leaves of all potato cultivars. Most isolates of pathogenic fungi (the species *Alternaria alternata* was isolated most frequently) were obtained from the leaves of cv. 'Adam' in the control treatment. The dominant species colonizing potato stems were *Colletotrichum coccodes* and *A. alternata*. Foliar fertilization had variable effects on the population size of pathogens colonizing the potato stem base. More pathogenic fungal isolates were obtained from stem base of potato plants grown in plots with a higher level of mineral fertilization.

Key words: potato stems, potato leaves, fungi, foliar fertilization, mineral fertilization

Introduction

The composition of fungal communities (including pathogens of the genus *Alternaria*) colonizing the aboveground parts of potato plants is affected, among others, by weather conditions, cultivars grown (Lenc 2007), crop rotation (Cwalina-Ambroziak and Czajka 2000), chemical and biological control (Kurzawińska and Mazur 2007) and soil mineral fertilization (Blachiński et al. 1996, Feng and

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Zheng 2006, Mac Donald et al. 2007). Foliar micronutrient fertilization plays an important role in potato growth and development under stress conditions (Malakouti 2008), provides protection against pathogens (Miller and Rosen 2005, Pavlista 2005) and influences the structure of pathogenic fungal communities colonizing potato leaves and stems (Cwalina-Ambroziak et al. 2007).

The objective of this study was to determine the composition of fungal communities colonizing the leaves and stem base of three potato cultivars in treatments with foliar applied micronutrient fertilizers and mineral soil fertilization.

Materials and methods

Three potato cultivars, medium-early 'Adam', medium-late 'Pasja Pomorska' and late 'Ślęza', were grown in a three-year plot experiment 2008–2010 (randomized split-plot design, three replications), established on grey-brown podsolic soil developed from light silty loam of complex 2, quality class IIIa, in Bałcyny, by the Department of Agrotechnology and Crop Production Management, University of Warmia and Mazury in Olsztyn. Certified seed potato tubers were planted in rows, 40 cm apart, at a row spacing of 62.5 cm. The first experimental factor was the level of mineral fertilization: A – 80 kg N per 1 ha, 80 kg P per 1 ha, 120 kg K per 1 ha, B – 120 kg N per 1 ha, 144 kg P per 1 ha, 156 kg K per 1 ha. The second experimental factor was foliar fertilization: a – Basfoliar 12-4-6 (8 $l \cdot ha^{-1}$), b – ADOB Mn (4 $l \cdot ha^{-1}$), c – Solubor DF (2 $l \cdot ha^{-1}$), d – ADOB Mn (2 $l \cdot ha^{-1}$) + Solubor DF (1 $l \cdot ha^{-1}$), e – ADOB Mn (2 $l \cdot ha^{-1}$) + Basfoliar 12-4-6 (4 $l \cdot ha^{-1}$), f – Basfoliar 12-4-6 (4 $l \cdot ha^{-1}$) + Solubor DF (1 $l \cdot ha^{-1}$), g – Basfoliar 12-4-6 (2.7 $l \cdot ha^{-1}$) + ADOB Mn (1.3 $l \cdot ha^{-1}$) + Solubor DF (0.7 $l \cdot ha^{-1}$), h – without foliar fertilization. Foliar fertilizers were applied once, at the beginning of flowering (BBCH 61). Cereal crops were grown as a forecrop. Identical plant protection measures, recommended by the Institute of Plant Protection – National Research Institute in Poznań, were implemented in all plots. Fungi were isolated from the leaves (according to Chruściak 1974) and stem base of potato plants at the laboratory. After flowering, 20 leaves were collected randomly from the middle layer of potato plants in plots of each treatment. 1 cm^2 samples of leaf tissue cut out at the base were placed in 200 ml flasks containing 90 ml sterile water. The flasks were shaken for 10 min and 0.2 ml suspension portions were transferred to Petri dishes containing PDA medium with rose Bengal and streptomycin. Stems collected four weeks before harvest were cut into 0.5 cm segments, which were disinfected with 50% ethylene and 1% sodium hypochlorite and treated with distilled water and placed on PDA. The cultured fungal colonies were inoculated onto agar slants for later microscopic identification according to the relevant keys (von Arx 1970, Ellis 1971, Nelson et al. 1983).

Temperature distribution patterns (May–August) were similar in the investigated growing seasons. Mean monthly temperatures were comparable with the long-term average in May and June, and they were higher than average in July and August (18°C to 21°C). Precipitation totals in the growing season of 2008 (226

mm) were lower than normal, and half of the rainfall occurred in August. The years 2009 and 2010 were wet (above-average precipitation totals of 331 mm and 366 mm, respectively), and particularly heavy rainfall events were observed in June 2009 and in May 2010.

Results and discussion

A more abundant community of fungi colonizing potato leaves (48 species of filamentous fungi, yeast-like fungi and non-sporulating cultures, Table 1) was isolated from late cultivars ('Pasja Pomorska' – 37.2% of all isolates, 'Ślęza' – 53.7%) than from the medium-early cultivar ('Adam' – 10%, Fig. 1 a). The above was due to the high abundance of yeast-like fungi on the leaves on late cultivars (Fig. 1 b). According to Chruściak (1974) and Kuczyńska (1992), yeast-like fungi are commonly found on the leaves of many plant species. Among filamentous fungi, the most abundant saprotrophic species was *Cladosporium cladosporioides*, while species of the genus *Humicola* (*H. brevis, H. fuscoatra*), the order *Mucorales* (*Mortierella alpina, M. isabellina, M. zonata, Mucor circinelloides, M. hiemalis*) and the genus *Penicillium* were isolated less frequently. The above fungal species were also isolated from potato leaves in a previous study (Cwalina-Ambroziak and Wróbel 2004). According to Kulikov et al. (2006), potato leaves were abundantly colonized by saprotrophic fungi of the genera *Mucor* and *Penicillium*, and by pathogenic fungi of the genera *Alternaria* and *Fusarium*. In the present study, pathogenic fungi had a low share in communities isolated from the leaves of the analyzed potato cultivars (10% in cv. 'Adam' and 2% in each of the remaining cultivars), and *A. alternata* was the most common species. Other pathogenic fungi, including *A. solani, Botrytis cinerea, C. coccodes*, eight species of the genus *Fusarium, Helminthosporium solani* and *Rhizoctonia solani*, were not abundant. The highest number of isolates of early blight causal agent were obtained in the rainfall-deficient growing season of 2008. According to Lenc (2007), the development of early blight is supported by long drought periods interspersed by showers. As demonstrated by Olanya et al. (2006), infections caused by the discussed pathogen are more common in the warm, summer months. In the present study, the causal agents of leaf diseases were isolated more frequently from potatoes of the medium-early cultivar fertilized with NPK at a lower level (A), in comparison with those fertilized at a higher level (B). A reverse trend was observed with respect to late cultivars. The effect of foliar fertilizers on the occurrence of pathogens in the fungal community colonizing potato leaves was most pronounced in cv. 'Adam', where pathogenic fungi accounted for 4.8% in the ADOB Mn + Solubor DF treatment to 18.6% in the control treatment (Fig. 2). In the remaining cultivars, the differences in the population size of pathogenic fungi in foliar fertilization treatments were insignificant. Mac Donald et al. (2007) reported that high N doses reduced the severity of potato infection by *Alternaria*.

Table 1

Fungi isolated from potato leaves in 2008–2010

Species	A								B							
	a	b	c	d	e	f	g	h	a	b	c	d	e	f	g	h
Alternaria spp.	52	94	66	64	38	56	64	33	73	50	31	63	36	77	40	46
Other pathogens (*B. cinerea*, *C. coccodes*, *Fusarium* spp., *H. solani*, *R. solani*)	12	20	14	9	18	10	2	13	12	8	12	8	6	41	23	17
Cladosporium cladosporioides	177	157	84	107	177	235	145	98	133	109	182	185	95	124	90	84
Other saprotrophic (*A. strictum*, *Humicola* spp., *Mucorales*, *Penicillium* spp.)	58	15	35	52	50	46	39	29	51	37	32	75	54	77	42	26
Non-sporulating	34	25	16	22	15	51	5	17	63	25	36	31	40	34	41	35
Yeast-like fungi	2 609	2 352	2 271	2 933	2 274	2 626	2 247	2 605	3 465	3 413	1 799	2 947	2 115	2 051	2 466	2 119
Other fungi*	115	86	82	110	71	79	65	36	65	51	46	72	82	89	49	65
Total	3 057	2 749	2 568	3 297	2 643	3 103	2 567	2 831	3 862	3 693	2 138	3 381	2 428	2 493	2 751	2 392

*Other fungi: *Arthrinium sphaerospermum, Ascochyta* spp., *Aspergillus* spp., *Aureobasidium pullulans, Chaetomium globosum, Coniothyrium* spp., *Endothia* spp., *Epicoccum* spp., *Monodictis glauca, Phialophora* spp., *Phoma* spp., *Pyrenochaeta* spp., *Sporotrichum olivaceum, Ulocladium botrytis.*

A, B – levels of mineral fertilization (A – 80 kg N per 1 ha, 80 kg P per 1 ha, 120 kg K per 1 ha, B – 120 kg N per 1 ha, 144 kg P per 1 ha, 156 kg K per 1 ha).

a, b, c, d, e, f, g, h – foliar fertilization (a – Basfoliar 12-4-6, b – ADOB Mn, c – Solubor DF, d – ADOB Mn + Solubor DF, e – ADOB Mn + Basfoliar 12-4-6, f – Basfoliar 12-4-6 + Solubor DF, g – Basfoliar 12-4-6 + ADOB Mn + Solubor DF, h – control, without foliar fertilization).

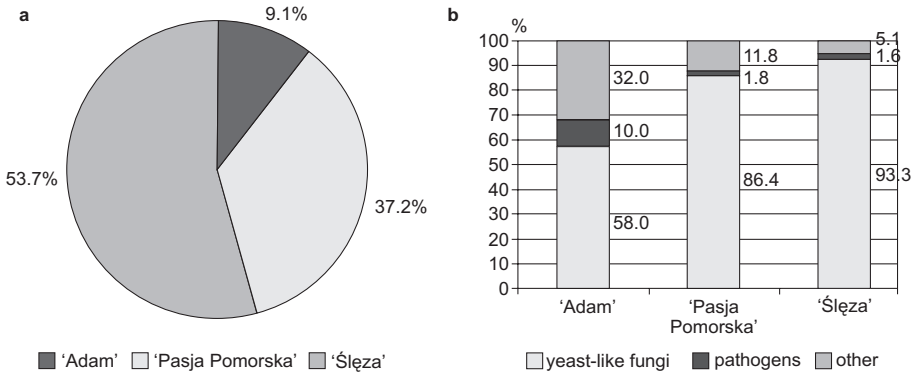
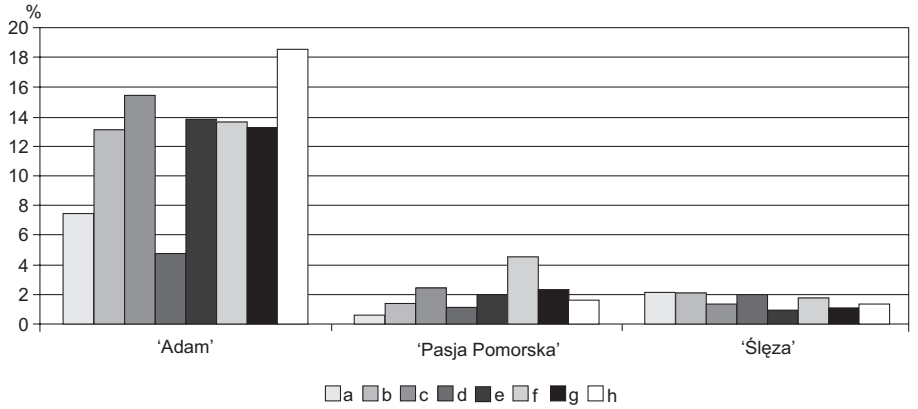


Fig. 1. Fungi isolated from potato leaves

The abundance of fungi (36 species of filamentous fungi, yeast-like fungi and non-sporulating cultures) colonizing the stems of the studied potato cultivars was similar (Table 2, Fig. 3 a). Pathogens were highly abundant in the fungal communities. The species identified most frequently was *C. coccodes* (approx. 52.6%), followed by *A. alternata* (29.5%, Fig. 3 b), similarly as in the case of leaves. *Fusarium* spp. were less common, while *B. cinerea, H. solani* and *R. solani* were isolated sporadically. The above species are considered typical potato stem pathogens (Cwalina-Ambroziak et al. 2007). Pathogen *C. coccodes* is known to infest potato stems, stolons and roots, but also tubers (Andrivon et al. 1998). Pathogenic fungi were more abundant (4% in the medium-early cultivar and 2% in late cultivars) in the treatment with a higher mineral fertilization level than with a lower mineral fertilization level (Fig. 4 a). The causal agent of anthracnose, in contrast to the causal agent of alternariosis, was more frequently isolated from potato stems in treatments with lower levels of mineral fertilization, compared with treatments



a, b, c, d, e, f, g, h – foliar fertilization (a – Basfoliar 12-4-6, b – ADOB Mn, c – Solubor DF, d – ADOB Mn + Solubor DF, e – ADOB MN + Basfoliar 12-4-6, f – Basfoliar 12-4-6 + Solubor DF, g – Basfoliar 12-4-6 + ADOB MN + Solubor DF, h – control)

Fig. 2. Pathogens isolated from potato leaves

Table 2

Fungi isolated from potato stems in 2008–2010

Species	A								B							
	a	b	c	d	e	f	g	h	a	b	c	d	e	f	g	h
Alternaria alternata	63	85	60	96	67	84	87	69	79	80	108	95	102	78	79	68
Colletotrichum coccodes	168	148	123	155	157	132	185	143	158	127	101	169	144	127	146	134
Fusarium spp.	39	11	5	17	17	9	15	11	12	17	14	10	14	30	31	19
Other pathogens (*B. cinerea, H. solani, R. solani*)	15	1	7	3	1	8	5	6	4	5	14	2	7	9	6	14
Antagonists (*Gliocladium* spp., *Paecilomyces* spp., *Trichoderma* spp.)	2	–	2	2	2	3	–	1	4	1	1	–	1	3	8	4
Mucorales	6	4	3	6	7	4	1	32	1	5	2	11	1	11	10	23
Other fungi*	22	26	47	15	18	22	10	3	7	15	13	6	17	8	10	11
Total	315	275	247	294	269	262	303	265	265	250	253	293	286	266	290	273

*Other fungi: *Acremonium strictum, Aureobasidium pullulans, Chaetomium globosum, Coniothyrium* spp., *Epicoccum* spp., *Gliomastix murorum, Humicola* spp., *Monodictis* spp., *Papulaspora irregularis, Phoma eupyrena, Scytalidium lignicola, Sporotrichum olivaceum.*

Explanations – as under Table 1.

with higher NPK levels. Zarzycka (1990) pointed to a better development of *C. coccodes* in nitrogen-deficient soil, in comparison with soil fertilized with adequate amounts of nitrogen. However, Feng and Zheng (2006) showed an inhibitory effect of potassium on the growth of *A. alternata*. The causal agent of anthracnose was isolated most frequently from the stems of potato cv. 'Pasja Pomorska' in the

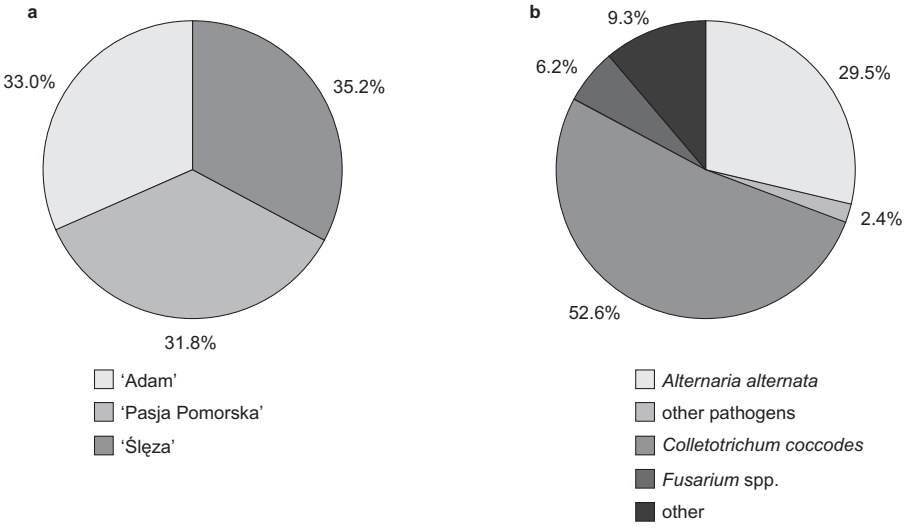


Fig. 3. Fungi isolated from potato stems

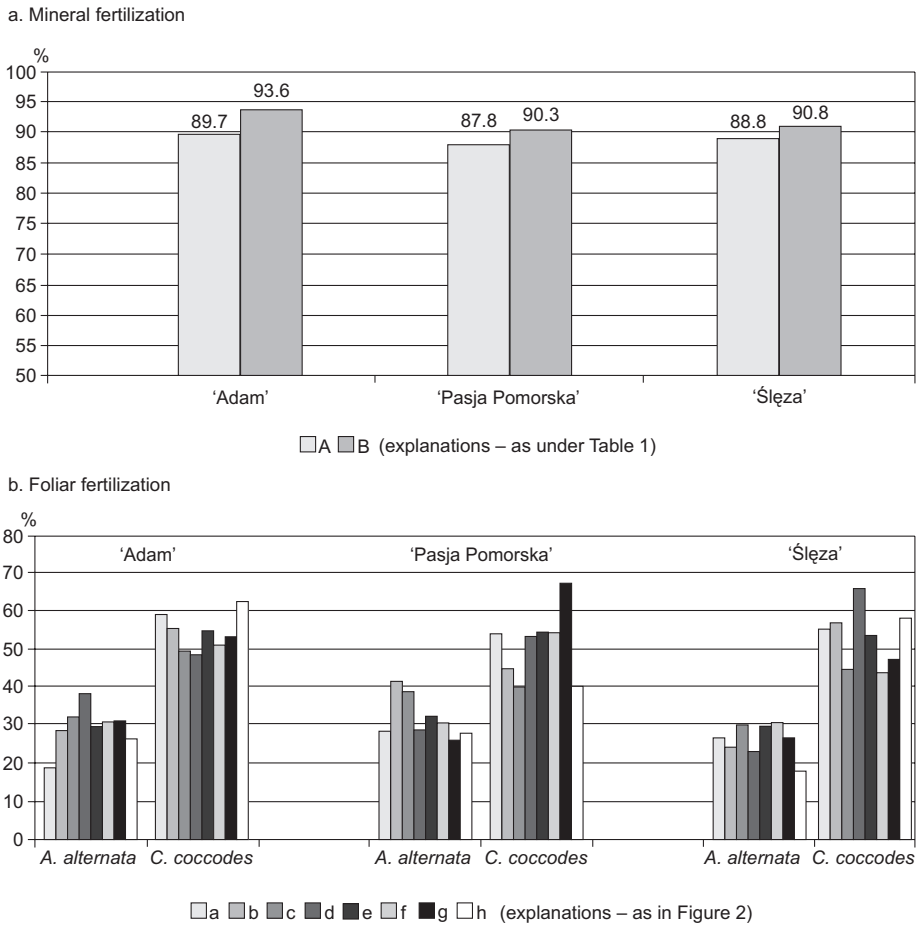


Fig. 4. Pathogens isolated from potato stems

treatment with three foliar fertilizers applied in combination, and from the stems of cv. 'Ślęza' in the ADOB Mn + Solubor DF treatment. *Alternaria alternata* had the highest share (approx. 40%) of fungal communities isolated from the medium-early cultivar in treatment "d" and from the medium-late cultivar in treatments "b" and "c". The lowest abundance of *A. alternata* was noted in fungal communities colonizing the stems of potato cv. 'Adam' and 'Pasja Pomorska' fertilized with Basfoliar 12-4-6, and the stems of non-fertilized cv. 'Ślęza' (Fig. 4 b). In a study by Osowski (2005), the growth of *Alternaria* fungi was inhibited by Basfoliar 12-4-6 applied in combination with the fungicides Antracol 70 WG and Unikat 75 WG. In a previous experiment, Cwalina-Ambroziak et al. (2007) noted the lowest abundance of *A. alternata* on the aboveground parts of potato plants treated with Basfoliar 12-4-6, ADOB Mn and Solubor DF. In this study, saprotrophs, including antagonists and fungi of the order *Mucorales*, were not isolated in great abundance. In an *in vitro* test, mineral fertilizers (ammonium chloride, am-

monium sulfate, single super phosphate, potassium chloride, diammonium phosphate, potassium sulfate and NPK) stimulated the growth, sporulation and antifungal activity of *T. viride* (Palanna et al. 2005).

It may be concluded that the leaves of the studied potato cultivars, in particular late cultivars, were abundantly colonized by yeast-like fungi. Pathogens had a 10% share of the fungal community isolated from the leaves of the medium-early potato cultivar (pathogens were isolated most frequently from treatments without foliar fertilizers), and a 2% share of fungal communities obtained from the leaves of both late cultivars. Pathogenic fungi accounted for approximately 90% in fungal communities colonizing potato stems, and the causal agents of anthracnose and early blight were identified most frequently. Pathogens were more frequently isolated from the stems of potato plants fertilized with a higher rate of mineral fertilizers, compared with a lower rate. Foliar fertilization had variable effects on the structure of fungal communities colonizing the aboveground parts of potato plants.

Streszczenie

PATOGENY NADZIEMNEJ CZĘŚCI ZIEMNIAKA NAWOŻONEGO DOLISTNIE

Badania prowadzono w celu określenia składu zbiorowiska grzybów kolonizujących liście i podstawę łodyg trzech odmian ziemniaka nawożonego NPK doglebowo (dwa poziomy nawożenia) i nawozami dolistnymi: Basfoliar 12-4-6, ADOB Mn i Solubor DF (aplikowanymi pojedynczo i w kombinacjach). Z liści badanych odmian najliczniej izolowano grzyby drożdżopodobne. Najwięcej patogenów (z najczęściej izolowanym gatunkiem *Alternaria alternata*) otrzymano z liści odmiany 'Adam' w kombinacji kontrolnej. Wśród grzybów kolonizujących łodygi ziemniaka dominujące były gatunki *Colletotrichum coccodes* i *A. alternata*. Nawożenie dolistne różnicowało liczebność grzybów patogenicznych kolonizujących łodygi ziemniaka. Więcej izolatów patogenów otrzymano z podstawy łodyg roślin na poletkach z wyższym poziomem nawożenia NPK niż z niższym.

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