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ORIGINAL PAPER

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WEEDS IN POTATO CULTURE AND THEIR OUTCOME IN SPREADING OF *Alternaria* spp.

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ABSTRACT

The aim of this study was to determine whether the weeds accompanying potato crops can be a source of *Alternaria* spp. causing Alternaria leaf blight and to determine the genetic similarities of *Alternaria alternata* isolates infecting selected weeds: *Chenopodium album*, *Cirsium arvense* and tested potato cultivar. Three-year field experiment was conducted on the potato cultivar 'Vineta N'. The isolates were classified into different species on the basis of macro- and microscopic features. In each year of the study, *A. alternata* dominated among the isolated fungi colonizing the leaves of potato plants and the selected weeds. The genetic similarities of *A. alternata* isolates was determined by the RAPD-PCR method. Tested genetic forms of *A. alternata* were closely related; only small differences in the pattern of the separated amplification products was evidenced. The dominance of *A. alternata* on the weeds accompanying potato crops suggests that if weed infestation is extensive, the pathogen is very likely to spread and its population to increase.

Key words: *Alternaria alternata*, *Chenopodium album*, *Cirsium arvense*, CTAB method, cultivar 'Vineta N'

INTRODUCTION

Global losses in agriculture have been estimated to around 35% of annual production due to abiotic and biotic factors. Among the biotic factors, phytopathogenic fungi are the major infectious agents in plants, producing diseases and/or toxic substances to human health [Larrañaga et al. 2012]. The Deuteromycetes fungal genus *Alternaria* comprises of different saprobiotic as well as endobiotic species and is well known for its notoriously destructive plant pathogen members [Mamgain et al. 2013]. The genus *Alternaria* contains different and all-over spread species of fungi, including aggressive and opportunistic plant pathogens affecting the majority of cultivated plants [Aradhya et al. 2001]. These fungi can affect crops in field or cause harvest and postharvest decay of plant products [Logrieco et al. 2009] of various species. All over the world, many authors report diseases caused by *Alternaria* spp. concerning: fruits – apples [Sofi et al. 2013], kiwi fruits [Yan et al. 2013], mandarin [Nemsa et al. 2012]; vegetables – carrot [Solfrizzo et al. 2005], onion [Shahnaz et al. 2013], tomato [Taskeen-Un-Nisa et al. 2011]; cereals – wheat [Perello and Larran 2013], barley [Kwaśna et al. 2006], rice [Iram and Ahmad 2005]; weeds [Siddiqui et al. 2011] and ornamental plants [Levy et al. 2006]. Approximately 30 metabolites with possible toxicity to humans and animals are known from various species of

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Alternaria. The most dangerous mycotoxins produced by the most widespread *A. alteranta* species include: alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxins I, II, III (ATX-I, II, III) and L-tenuazonic acid (TEA) [Lee et al. 2015].

In terms of global production, potato is the fourth most important nutritional crop plant in the world. According to FAO [Food and Agriculture Organization 2013], the total worldwide area under potato cultivation in 2013 was estimated at 19,463,041.08 hectares, with a global production of 368,096,362.22 tons per year. Development of some pathogens and diseases caused by them on potato plants results in yield losses of economic importance [Kurzawińska and Mazur 2012].

Apart from potato blight, the most serious disease that produces damage to the plant's assimilation surface is Alternaria blight caused by *Alternaria alternata* (Fries, Keissler) and *Alternaria solani* (Sorauer). This common disease, that can be found in most potato-growing countries, can cause considerable defoliation [Woudenberg et al. 2014]. The harmfulness of this disease is particularly high because of the weather conditions favorable to its development and rising losses caused by its early occurrence (bud-formation stage and flowering). The growth and development of weeds is faster than crop plants, therefore also the development of some pathogens on them will be faster. The threat of wild-growing plants that are infected earlier may be large due to the increase of infection potential of the pathogen. Ubiquitous fungi of the genera *Alternaria* can infect previously developed weeds faster and faster attack crops, causing larger loss in the quality and quantity of yield. It is important to determine the threat from weeds as a source of spores threatening *Alternaria* spp. for potato. The literature data indicates that among the likely hosts to some *Alternaria* species are: *Amaranthus retroflexus* L. [Pusz 2009; Mazur et al. [2015], *Chenopodium album* L. [Siddiqui et al. 2010, 2011] and *Cirsium arvense* (L.) Scop. [Hongmei et al. 2011].

The aim of the present study conducted at the Mydlniki Experimental Station was to determine whether the weeds accompanying potato crops could be a source of *Alternaria* spp. that cause Alternaria leaf blight and to determine genetic diversity of the isolates infecting the selected weeds and tested potato cultivar.

MATERIAL AND METHODS

Plant material

Three-year field experiments (2014–2016) were conducted on the potato cultivar 'Vineta N' in the Mydlniki Experimental Station of the Department of Vegetable and Herb Plants – University of Agriculture in Kraków (50°04'46"N, 19°50'48"E). This cultivar present sensitive leaves and quite susceptible tubers to potato late blight and present a good storage durability and moderate resistance to mechanical damage [Eremeev et al. 2006].

Weed species accompanying the potato crops in this experiment were selected for mycological tests. These were: *Chenopodium album* L., and *Cirsium arvense* L.) Scop. On their leaves round, sometimes angular, dark-brown spots were presented. The first symptoms of weed infestation by *Alternaria* spp. were observed at the beginning or the middle of June, the term depended on the weather conditions. Initially symptoms were noticed on several plants, at the end of June the symptoms were observed on most weeds. The latest and the smallest severity of the disease was in 2015, due to prolonged drought, small amount of rainfall and low air humidity. The plots were not protected by fungicides. The stains on the potatoes were larger than on the weeds, the yellow borders around the stains were clearer. This was caused by the secretion of toxic secondary metabolites by *Alternaria* spp. destroying plant cells.

The leaf fragments showing obvious disease symptoms, after surface disinfection and drying between layers of filter paper next were located on the PDA medium.

Fungal material

The growing colonies of fungi obtained from the weed leaves were transplanted and subjected to continued incubation. Identification of fungi was made on the basis of an assessment of the colonies and conidiospores [Dugan 2006, Klaus et al. 2008]. In the description of the fungal communities from the leaves, they were divided into:

dominant (more than 5%), influential (1–5%), and accessory (below 1%).

DNA extraction

Each year of the study, three isolates of *A. alternata* were selected for the genetic tests − two from tested weeds (*Chenopodium album* and *Cirsium arvense*) and one from potato leaves. Concerning selected isolates, homosporous cultures were obtained, from which DNA was subsequently extracted. The extraction of genomic DNA from the isolates was performed by CTAB method [Gardes and Bruns 1993], as follows: 300 µl 2× CTAB lysis buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA, pH 8.0; 2% CTAB; 0.2% β-mercaptoethanol) was added to 1.5 ml reaction tubes containing fresh mycelium collected from homosporous cultures. The samples suspended in the buffer were frozen in liquid nitrogen, slightly thawed and crushed with a micropestle. The samples were incubated at 65°C for 30 min at a heat block. One volume of chloroform: isoamyl alcohol (24 : 1) was added to each sample and mixed by briefly vortexing. The samples were centrifuged for 15 min at 14 000 rpm and room temperature. The aqueous phase was collected from each tube and transferred to a new reaction tube. The equal volume of cold isopropyl alcohol was used to precipitate the DNA from each sample. After 10 min, the centrifugation (10 min at 140 000 rpm) was performed to pellet the precipitant. The supernatant was discarded and the pellet was washed with 70% ice-cold ethanol solution. In the final step, the pellet was re-suspended in 50 µl of 0.1× TE buffer (1 mM Tris-HCl, pH 8.0; 0.1 M EDTA, pH 8.0). The quantification of isolated DNA samples was performed by 1.5% agarose gel electrophoresis using MassRuler DNA Ladder Mix (Fermentas) as a mass standard. Upon the quantification results, the samples were diluted in a sterile double-distilled water to obtain the final concentration of 20 ng/µl.

PCR amplification. RAPD-PCR amplification of nine *A. alternata* DNA samples was performed using OPAD12 primer (5'-AAGAGGGCGT-3') and Fermentas™ reagents. The reaction mixture contained: approximately 20 ng of DNA template, 0.4 µM OPAD12 primer, 0.1 mM dNTPs mix, 1.5 mM $MgCl_2$, 0.5 U *Taq* DNA polymerase and 1× *Taq* Buffer with $(NH_4)_2SO_4$™ (750mM Tris-HCl, pH 8.8; 200 mM $(NH_4)_2SO_4$; 0.1% Tween 20). The PCR conditions in the C 1000 Bio Rad thermocycler consisted of an initial denaturing at 95°C for 3 min, followed by 45 cycles of: 94°C for 45 s, 38°C for 45 s, 72°C for 45 s and a final elongation at 72°C for 10 min and then cooled and held at 4°C. RAPD-PCR products were separated in 1.5% agarose gel electrophoresis in 1× TBE buffer and visualized by ethidium bromide staining.

Data analysis

Polymorphism was scored on a basis of bands presence or absence and data were analyzed using the NTSYS-PC software. Genetic similarity between tested isolates was determined using unweighted pair group method with arithmetic mean (UPGMA).

RESULTS AND DISCUSSION

Overall, from the necrotic spots on the potato leaves and tested weed plants, 1290 fungal colonies were isolated. These isolates belonged to 18 species, represented by 15 genera. The most numerous among them were fungi of the genus *Alternaria*. The fungi isolated in the dominant species group were those of the genus *Alternaria* (Tabs. 2−4), including *A. alternata* and *A. solani*. Classified into the dominant species, there were also: *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *Cladosporium herbarum* (Pers.) Link, *Epicoccum nigrum* Link, and *Fusarium roseum* Link. Other species were less numerous; they were likely to have colonized tissues that had already been infected.

The percentage share of *A. alternata* isolates ranged from 20.0 to 28.2% (Fig. 1). The largest number of isolates of this fungus was obtained in the third year (higher average temperature of 18.6°C in June, drought). By contrast, fewer isolates of *A. alternata* were obtained in the second year, which could have been associated with a high level of rainfall in July and August; the total rainfall was 112.4 and 138.2 mm, respectively (Tab. 1).

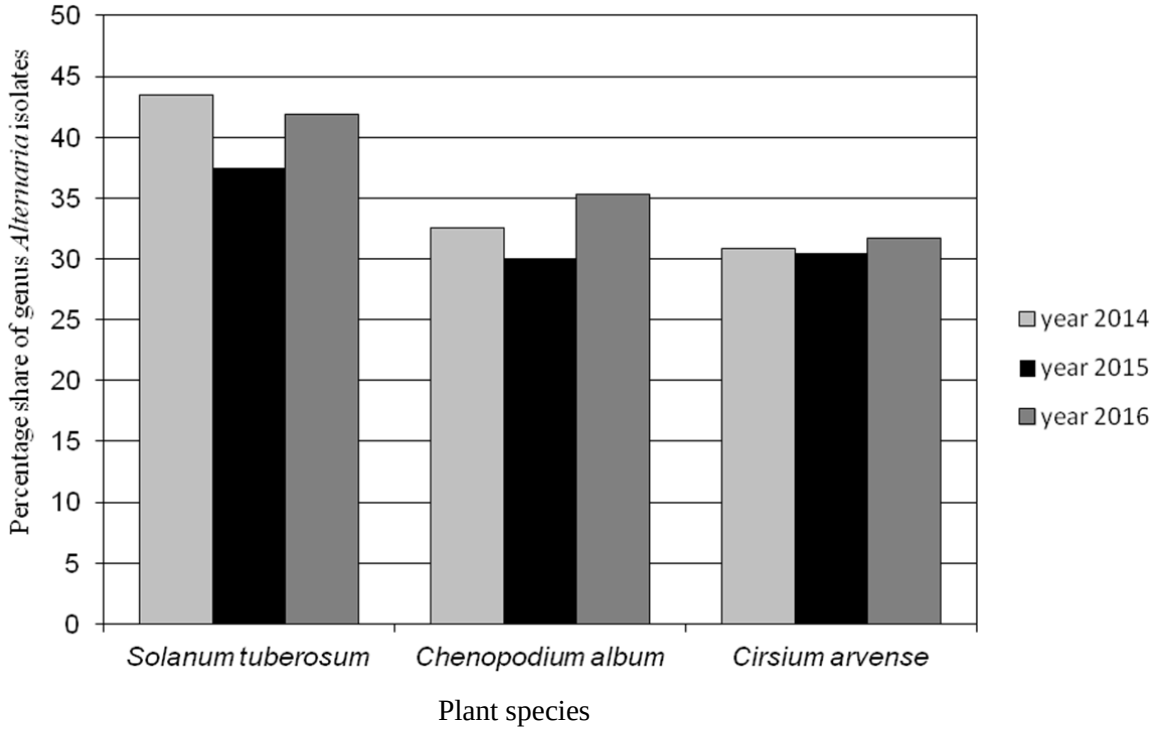

Fig. 1. Percentage share of genus *Alternaria* isolated from the potato leaves and weeds in 2014–2016

Table 1. Weather conditions during the growing season in Mydlniki (Poland) during three experimental years

Experimental year	Month	Average air temperature (°C)				Rainfall sum (mm)			
		for decade				for decade			
		I	II	III	monthly	I	II	III	monthly
First year	May	12.9	13.1	13.6	13.2	0.2	58.4	65.3	123.9
	June	13.7	15.3	17.9	15.6	31.0	62.6	121.6	215.2
	July	19.4	19.2	19.4	19.3	61.5	45.3	40.3	147.1
	August	19.3	18.4	17.3	18.3	1.3	12.3	56.6	59.8
Second year	May	12.7	10.9	14.5	12.7	79.4	188.0	35.0	302.4
	June	18.6	17.9	16.9	17.8	1.6	28.0	22.0	51.6
	July	19.8	23.6	21.3	21.6	8.6	31.6	72.2	112.4
	August	19.9	19.6	18.6	19.4	27.8	38.0	72.4	138.2
Third year	May	9.6	15.4	17.2	14.1	16.0	12.2	26.4	54.6
	June	19.4	18.4	17.9	18.6	26.0	29.6	11.4	67.0
	July	17.3	20.1	16.4	17.9	45.0	62.2	55.8	163.0
	August	18.7	19.2	20.3	19.4	23.0	5.8	8.4	37.2

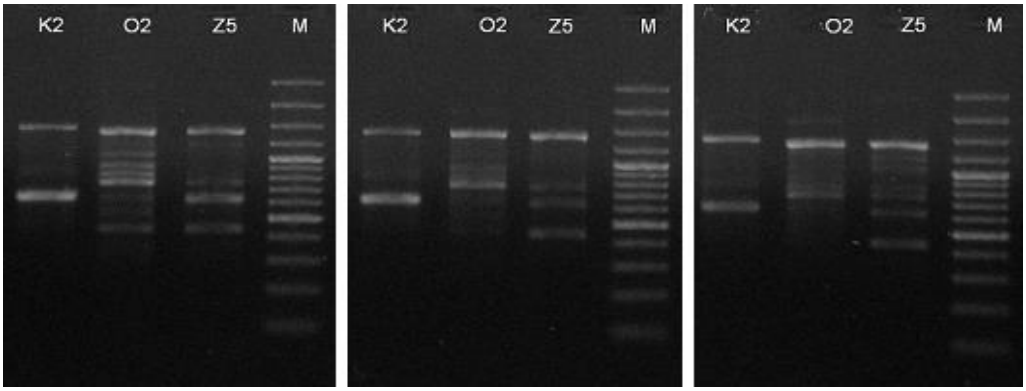

Fig. 2. Image of the electrophoretic division of the DNA of *Alternaria* isolated from the potato leaves and tested weeds: *Chenopodium album* (K2), *Cirsium arvense* (O2), *Solanum tuberosum* (Z5), markers (M) in years 2014–2016

Table 2. Quantitative composition of the fungi isolated from the potato leaves and selected weeds in 2014

Species of fungi	*Solanum tuberosum*		*Chenopodium album*		*Cirsium arvense*	
	number of isolates	percentage of isolates	number of isolates	percentage of isolates	number of isolates	percentage of isolates
Alternaria alternata	57	28.2	18	20.9	16	20.5
Alternaria botrytis	2	1.0	1	1.2	2	2.6
Alternaria solani	31	15.3	10	11.6	8	10.3
Aspergillus spp.	0	0.0	2	2.3	3	3.8
Botrytis cinerea	6	3.0	4	4.7	3	3.8
Cladosporium cladosporioides	21	10.4	17	19.8	14	17.9
Cladosporium herbarum	22	10.9	0	0.0	5	6.4
Colletotrichum coccodes	5	2.5	0	0.0	2	2.6
Dichotomopilus indicus	0	0.0	2	2.3	2	2.6
Epicoccum nigrum	11	5.4	8	9.3	7	9.0
Fusarium caeruleum	6	3.0	0	0.0	0	0.0
Fusarium roseum	10	5.0	6	7.0	4	5.1
Hyalocylindrophora rosea	4	2 .0	7	8.1	2	2.6
Juxtiphoma eupyrena	7	3.5	0	0.0	0	0.0
Sordaria fimicola	4	2.0	2	2.3	2	2.6
Stemphylium botryosum	5	2.5	0	0.0	2	2.6
Trichoderma viride	5	2.5	2	2.3	2	2.6
Trichothecium roseum	6	3.0	7	8.1	4	5.1
Total	202	100.0	86	100.0	78	100.0

Table 3. Quantitative composition of the fungi isolated from the potato leaves and selected weeds in 2015

Species of fungi	*Solanum tuberosum*		*Chenopodium album*		*Cirsium arvense*	
	number of isolates	percentage of isolates	number of isolates	percentage of isolates	number of isolates	percentage of isolates
Alternaria alternata	43	20.4	14	20.0	12	20.3
Alternaria botrytis	1	0.5	2	2.9	1	1.7
Alternaria solani	36	17.1	7	10.0	6	10.2
Aspergillus spp.	0	0.0	3	4.3	2	3.4
Botrytis cinerea	9	4.3	2	2.9	3	5.1
Cladosporium cladosporioides	27	12.8	15	21.4	11	18.6
Cladosporium herbarum	29	13.7	0	0.0	6	10.2
Colletotrichum coccodes	4	1.9	0	0.0	2	3.4
Dichotomopilus indicus	0	0.0	0	0.0	2	3.4
Epicoccum nigrum	14	6.6	7	10.0	5	8.5
Fusarium caeruleum	9	4.3	0	0.0	0	0.0
Fusarium roseum	16	7.6	4	5.7	2	3.4
Hyalocylindrophora rosea	7	3.3	9	12.9	2	3.4
Juxtiphoma eupyrena	4	1.9	0	0.0	0	0.0
Sordaria fimicola	2	0.9	0	0.0	1	1.7
Stemphylium botryosum	3	1.4	0	0.0	1	1.7
Trichoderma viride	3	1.4	1	1.4	1	1.7
Trichothecium roseum	4	1.9	6	8.6	2	3.4
Total	211	100.0	70	100.0	59	100.0

Table 4. Quantitative composition of the fungi isolated from the potato leaves and selected weeds in 2016

Species of fungi	*Solanum tuberosum*		*Chenopodium album*		*Cirsium arvense*	
	number of isolates	percentage of isolates	number of isolates	percentage of isolates	number of isolates	percentage of isolates
Alternaria alternata	50	27.2	16	23.5	14	22.2
Alternaria botrytis	3	1.6	1	1.5	1	1.6
Alternaria solani	27	14.7	8	11.8	6	9.5
Aspergillus spp.	0	0.0	1	1.5	2	3.2
Botrytis cinerea	7	3.8	3	4.4	4	6.3
Cladosporium cladosporioides	20	10.9	11	16.2	10	15.9
Cladosporium herbarum	24	13.0	0	0.0	5	7.9
Colletotrichum coccodes	4	2.2	0	0.0	4	6.3
Dichotomopilus indicus	0	0.0	1	1.5	0	0.0
Epicoccum nigrum	9	4.9	8	11.8	6	9.5
Fusarium caeruleum	6	3.3	0	0.0	0	0.0
Fusarium roseum	7	3.8	5	7.4	2	3.2
Hyalocylindrophora rosea	6	3.3	7	10.3	2	3.2
Juxtiphoma eupyrena	6	3.3	0	0.0	0	0.0
Sordaria fimicola	3	1.6	1	1.5	1	1.6
Stemphylium botryosum	2	1.1	0	0.0	2	3.2
Trichoderma viride	5	2.7	0	0.0	1	1.6
Trichothecium roseum	5	2.7	6	8.8	3	4.8
Total	184	100.0	68	100.0	63	100.0

The disease typically reduces yields by ~20%, but yield reductions of up to 80% have been reported [Horsfield et al. 2010]. In each year of the study, *A. alternata* dominated among the isolated fungi colonizing the potato leaves and weed plants in the experiments. Studies of the genus *Alternaria* are increasingly based on genetic analyses. Molecular biology methods make it possible to explore the genetic interrelations between isolates or groups of isolates, which cannot be determined with conventional tests [Cooke et al. 1998, Sharma and Tewari 1998]. Such information could lead to the development of more reliable methods for improving the potato breeding programs against early blight disease [El Komy et al. 2012].

Regarding the genetic analyses in 2015, considerable similarities (over 60%) were observed between isolates obtained from potato and *Cirsium arvense*. In the following years of the study, in the same group of the plants, there were also considerable similarities with slightly different values, but all above 60%. The similarity between isolates obtained from potato and *Chenopodium album* in three years of the study was less than 40%. The current results clearly indicate similarities between *A. alternata* isolates obtained from potato plants and those obtained from some weeds (Fig. 2), which proved the importance of wild plant communities as a source of the pathogen for potato crops.

The climate warming provides a better environment for the existence of *A. alternata* on plants, belonging to different botanical families. This is supported by the isolation of this species from weeds that most frequently accompanied the potato crops in the present study. High density of these plants and infection of at least one of them by that fungal species may lead to the spreading of the pathogen and an increase in its population size. The healthiness of potato leaves was better on weed plots, compared to plots where weeds were always present. The lowest intensity of Alternaria leaf blight, both on weeds and on leaves of potato, was noted in 2015, when during the growing season there was very little rainfall and even at nights there was little humidity. These conditions were clearly not conducive to infection of the pathogen. In order not to disturb the results, the plants in the experimental plots were not protected by fungicides.

CONCLUSIONS

During the study, each year *Alternaria alternata* was dominant among the fungi isolated from infected potato leaves and selected weeds. *A. alternata* weed's infestation, accompanying potato culture, pointed a constant threat for potato plants by this pathogen.

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