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Redroot pigweed as a host for *Alternaria alternata* – the causal agent of Alternaria leaf blight in potato

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Abstract

The aim of the study was to establish whether the redroot pigweed (*Amaranthus retroflexus* L.), which is a common weed in potato crops can be a source of *Alternaria alternata*, the causal agent of Alternaria leaf blight, and to determine the genetic diversity of isolates of this pathogen infecting the weed and the potato cultivar tested. With the *A. alternata* isolates selected for genetic testing, homosporous cultures were obtained, from which DNA (deoxyribonucleic acid) was subsequently isolated. The genetic diversity of *A. alternata* isolates was determined by the RAPD-PCR (random amplification of polymorphic-polymerase chain reaction) method. Based on the results obtained, it was found that the dominant fungi present on the diseased leaves of both potato and pigweed plants were: *Alternaria alternata*, *A. solani*, *Cladosporium cladosporioides*, *C. herbarum*, *Epicoccum purpurascens* and *Fusarium sambucinum*. Presence of *A. alternata* as a dominant fungus on redroot pigweed suggests that if weed infestation is extensive, the pathogen is very likely to spread and its population to increase.

Key words: *Alternaria alternata*, *Amaranthus retroflexus*, *Solanum tuberosum*.

Introduction

In spite of the decrease in the potato cultivation area, the production still has great economic weight (Kurzawińska, Mazur, 2008). One of the most important pathogens of potato, besides *Phytophthora infestans* (Mont de Bary), is *Alternaria alternata* (Fr.) Keissler. This fungus, along with another species of the genus *Alternaria* – *A. solani* (Sorauer), causes Alternaria leaf blight in potato, which is considered by many authors to be the most serious disease destroying the assimilation surface of plants. The disease is common in all areas of potato cultivation (Johnson et al., 2008, Kurzawińska, Mazur, 2009). The knowledge of soil environment where plants grow plays a considerable role in providing its advantageous features to assure satisfactory health state of plants, which is beneficial to increase the yield (Kurzawińska, 2006). The disease has become particularly harmful because the existing weather conditions are favourable for its development and the early onset of the disease causes higher yield losses. In Poland, the first symptoms of the disease have been observed on average 55–70 days after planting, which means that they appear most often in June, in particular in the second and third decade of the month (Kapsa, Osowski, 2007). According to Treikale et al. (2008), the resulting yield losses range from 6% to 45%. Weeds often found in potato crops can be hosts for certain species of fungi of the genus *Alternaria*. Among those weeds is the redroot pigweed (*Amaranthus retroflexus* L.) (Montazeri et al., 2005; Pusz, 2009; Uremis et al., 2009; Kahramanoglu, Uygur, 2010; Reza Haj Seyed Hadi, Noormohamadi, 2012; Kahramanoglu, 2014). An attempt was therefore made to establish whether the plants of pigweed occurring in potato crops can be a source of *A. alternata*, the causal agent of Alternaria leaf blight, and to determine the genetic diversity of the *A. alternata* isolates infecting the weed and the potato tested.

Materials and methods

A three-year field experiment was conducted on the potato (*Solanum tuberosum* L.) cultivar 'Vineta N' at the Agricultural University's Experimental Research Station in Kraków-Mydlniki in 2009–2011. The leaves of this cultivar are very sensitive and quite susceptible to late blight and features good storage durability and moderate resistance to mechanical damage (Eremeev et al., 2006). Winter wheat was used as a forecrop. Manure at a dose of 30 t ha^{-1}, triple superphosphate 46% P_2O_5 (80 kg ha^{-1}) and potassium chloride 60% K_2O (170 kg ha^{-1}) were applied in the autumn. Nitro chalk 27% N at 200 kg ha^{-1} was applied before planting in the spring. The experiment was set up spacing 62.5 cm between rows in April (24 04 2009, 23 04 2010, 22 04 2011). After ridging of potatoes, spraying was carried out as recommended with the herbicide Sencor 70 WG (a.i. metribuzin). Treatments were used in accordance with the standard agricultural practices.

Roundish brown spots, 2–5 mm in diameter, were found on the surface of the leaves of the potato and pigweed plants. Small fragments of the leaves showing obvious disease symptoms were selected for mycological tests. They were surface-disinfected with 70% ethanol, washed twice in sterile distilled water, dried with filter paper, and plated on the PDA (potato dextrose agar) medium. After an incubation period of 4–7 days at 22°C, the grown colonies were split off onto slants also containing the PDA medium. After development of the fungal isolates on the slants and macro- and microscopic examinations, representatives of the communities were selected. These isolates were inoculated into suitable media and identified with the help of mycological keys and monographs (Dugan, 2006; Klaus et al., 2008). In the characterization of the fungal communities from the leaves of potato and pigweed plants they were classified as: dominant (the most numerous), influential (moderately numerous) and accessory (not numerous) fungi. The criteria, adopted from Kowalik (2013), were based on the percentage of fungi in the entire community: >5% – dominant, 1–5% – influential, <1% – accessory fungi.

The *Alternaria alternata* isolates selected for genetic testing were used to produce homosporous cultures, from which DNA (deoxyribonucleic acid) was subsequently extracted. Extraction of genomic DNA from the isolates of the pathogen was carried out by the CTAB (cetyltrimethylammonium bromide) method (Gardes, Bruns, 1993).

Results and discussion

Necrotic spots of the tested plants were colonized by a population of 18 species of fungi (Tables 1–2). The dominant species in all experimental years in the communities of fungi isolated from the leaves

Table 1. Quantitative composition of the fungi isolated from potato leaves

Species of fungi	Number of isolates				Percentage of isolates			
	2009	2010	2011	Total	2009	2010	2011	Average
Acremonium roseum Petch	4	7	6	17	2.0	3.3	3.3	2.8
Alternaria alternata (Fr.) Keissl.	57	43	50	150	28.2	20.4	27.2	25.1
Alternaria solani Sorauer	31	36	27	94	15.3	17.1	14.7	15.7
Aspergillus spp.	0	0	0	0	0.0	0.0	0.0	0.0
Botrytis cinerea Pers.	6	9	7	22	3.0	4.3	3.8	3.7
Chaetomium indicum Corda	0	0	0	0	0.0	0.0	0.0	0.0
Cladosporium cladosporioides (Fresen.) G.A. de Vries	21	27	20	68	10.4	12.8	10.9	11.4
Cladosporium herbarum (Pers.) Link ex Gray	22	29	24	75	10.9	13.7	13.0	12.6
Colletotrichum coccodes (Wallr.) Hughes	5	4	4	13	2.5	1.9	2.2	2.2
Epicoccum nigrum Link.	11	14	9	34	5.4	6.6	4.9	5.7
Fusarium coeruleum (Saccardo) Booth	6	9	6	21	3.0	4.3	3.3	3.5
Fusarium sambucinum Fuckel	10	16	7	33	5.0	7.6	3.8	5.5
Phoma eupyrena Sacc.	7	4	6	17	3.5	1.9	3.3	2.8
Sordaria fimicola Rob.	4	2	3	9	2.0	0.9	1.6	1.5
Stemphylium botryosum Wallroth	5	3	2	10	2.5	1.4	1.1	1.7
Trichoderma viride Pers. ex Gray	5	3	5	13	2.5	1.4	2.7	2.2
Trichothecium roseum Link	6	4	5	15	3.0	1.9	2.7	2.5
Ulocladium botrytis Preuss	2	1	3	6	1.0	0.5	1.6	1.0
Overall	202	211	184	597	100.0	100.0	100.0	100.0

Table 2. Quantitative composition of the fungi isolated from redroot pigweed leaves

Species of fungi	Number of isolates				Percentage of isolates			
	2009	2010	2011	Total	2009	2010	2011	Average
Acremonium roseum Petch	3	2	2	7	2.9	2.7	2.2	2.6
Alternaria alternata (Fr.) Keissl.	21	17	20	58	20.0	23.0	22.2	21.6
Alternaria solani Sorauer	14	10	12	36	13.3	13.5	13.3	13.4
Aspergillus spp.	2	2	1	5	1.9	2.7	1.1	1.9
Botrytis cinerea Pers.	5	3	5	13	4.8	4.1	5.6	4.8
Chaetomium indicum Corda	2	1	2	5	1.9	1.4	2.2	1.9
Cladosporium cladosporioides (Fresen.) G.A. de Vries	19	13	14	46	18.1	17.6	15.6	17.1
Cladosporium herbarum (Pers.) Link ex Gray	9	7	7	23	8.6	9.5	7.8	8.6
Colletotrichum coccodes (Wallr.) Hughes	0	0	0	0	0.0	0.0	0.0	0.0
Epicoccum nigrum Link.	9	7	9	25	8.6	9.5	10.0	9.3
Fusarium coeruleum (Saccardo) Booth	2	0	0	2	1.9	0.0	0.0	0.7
Fusarium sambucinum Fuckel	7	6	7	20	6.7	8.1	7.8	7.4
Phoma eupyrena Sacc.	0	0	0	0	0.0	0.0	0.0	0.0
Sordaria fimicola Rob.	3	2	4	9	2.9	2.7	4.4	3.3
Stemphylium botryosum Wallroth	0	0	0	0	0.0	0.0	0.0	0.0
Trichoderma viride Pers. ex Gray	2	0	1	3	1.9	0.0	1.1	1.1
Trichothecium roseum Link	5	3	4	12	4.8	4.1	4.4	4.5
Ulocladium botrytis Preuss	2	1	2	5	1.9	1.4	2.2	1.9
Overall	105	74	90	269	100.0	100.0	100.0	100.0

of both potato and pigweed plants were: *Alternaria alternata* (from 20.4% to 28.2% of isolates of potato and from 20.0% to 23.0% of isolates of pigweed), *A. solani* (from 14.7% to 17.1% of isolates of potato and from 13.3% to 13.5% of isolates of pigweed), *Cladosporium cladosporioides* (from 10.4% to 12.8% of isolates of potato and from 15.6% to 18.1% of isolates of pigweed), *C. herbarum* (from 10.9% to 13.7% of isolates of potato and from 7.8% to 9.5% of isolates of pigweed).

The most frequent species in all years included also the fungi *Epicoccum nigrum* and *Fusarium sambucinum* occurring in much smaller numbers from 5.5% to 9.3%. Other species of the isolated fungi constituted from 1.0% to 4.3%. It needs to be emphasized that the leaves of the potato and pigweed plants tested were colonized to the greatest extent by *A. alternata*.

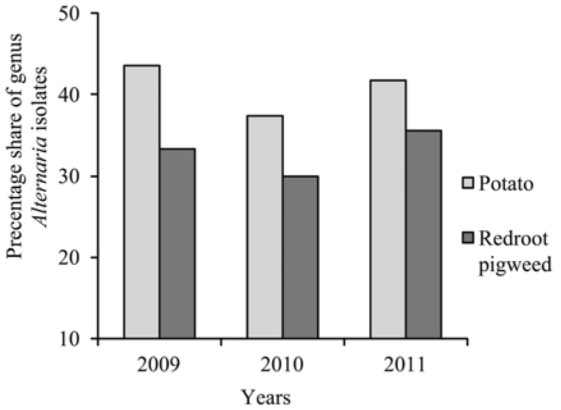

Figure 1. Percentage share of genus *Alternaria* isolated from leaves of potato and redroot pigweed in 2009–2011

Potato leaf colonization by fungi genus *Alternaria* in all experimental years was higher compared to that of redroot pigweed leaves (Fig. 1).

The occurrence of Alternaria blight depends on a complex of factors (Iglesias et al., 2007; Hubballi et al., 2010). According to Johnson et al. (2008) and Kapsa (2008), the development of epiphytoses of the disease is determined by, among other things, the average temperature in July–August above 18°C, relative air humidity up to 80%, heavy showers, overnight dew, and sandy soils.

Studies of the genus *Alternata* are increasingly based on genetic analyses. Molecular biology methods make it possible to explore the genetic relationships between isolates or groups of isolates, which cannot be determined by conventional methods (Gherbawy, 2005; Kale et al., 2012). Based on the results of the analyses presented here, it was found that in three cases there was high similarity between the DNA of the pathogen isolated from potato plants and that isolated from pigweed plants (Fig. 2). Evidence of the dominance of *A. alternata* on redroot pigweed, which is a common weed in potato crops, suggests that with extensive weed infestation an increase in the size of this pathogen's population may occur and the pathogen is likely to spread.

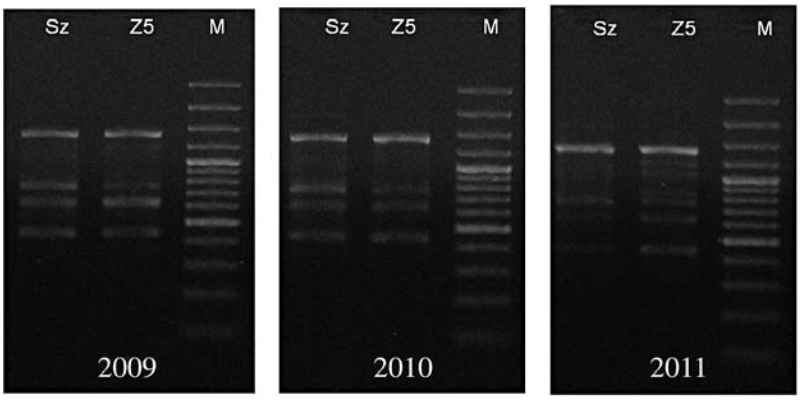

Figure 2. Image of the electrophoretic mobility of the DNA of *Alternaria alternata* isolated from the redroot pigweed (Sz), potato (Z5) and markers (M) in 2009–2011

Conclusions

1. The dominant fungi present on the diseased leaves of both potato and redroot pigweed plants were: *Alternaria alternata*, *A. solani*, *Cladosporium cladosporioides*, *C. herbarum*, *Epicoccum nigrum* and *Fusarium sambucinum*. The leaves of the tested plants were colonized to the largest extent by *Alternaria alternata*.

2. The similarities between *A. alternata* isolates obtained from potato plants and those obtained from pigweed plants signify the importance of weeds as a source of the pathogen for potato crops.

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Šiurkštusis burnotis – bulvių lapų sausligės sukėlėjo *Alternaria alternata* augalas šeimininkas

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Santrauka

Tyrimo tikslas – nustatyti, ar šiurkštusis burnotis (*Amaranthus retroflexus* L.), kuris yra bulvių pasėliuose paplitusi piktžolė, gali būti *Alternaria alternata*, bulvių lapų sausligės sukėlėjo, šaltinis, ir nustatyti šio patogeno, infekuojančio ir piktžolę, ir tirtos veislės bulves, genetinę įvairovę. *A. alternata* izoliatus surinkus genetiniam tyrimui buvo gautos homosporinės kultūros, iš kurių buvo išskirta DNR (deoksiribonukleo rūgštis). Genetinė *A. alternata* izoliatų įvairovė buvo nustatyta AAPD-PGR (atsitiktinai amplifikuotų polimorfinės DNR polimerazės grandininės reakcijos) metodu. Remiantis tyrimo rezultatais nustatyta, kad ant šiurkščiojo burnočio ir bulvių ligos pažeistų lapų aptikti vyraujantys grybai buvo šie: *Alternaria alternata*, *A. solani*, *Cladosporium cladosporioides*, *C. herbarum*, *Epicoccum purpurascens* ir *Fusarium sambucinum*. *A. alternata* dominavimas ant šiurkščiojo burnočio rodo, kad jeigu piktžolėtumas yra didelis, šis grybas gali išplisti, o jo populiacija padidėti.

Reikšminiai žodžiai: *Alternaria alternata*, *Amaranthus retroflexus*, *Solanum tuberosum*.