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FUNGI FROM SEEDS OF *AMARANTHUS* SPP.

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Abstract

Seeds of *Amaranthus cruentus, A. paniculatus* and *A. retroflexus* grown in Poland were colonized at least by 18 species of fungi. *Alternaria alternata* was the most common species. Its frequency in the fungal community amounted to 85%. The high levels of internal infection indicate that *A. alternata* was not a casual, random contaminant, but that its presence resulted from direct colonization of flower or seed. *Epicoccum nigrum* and *Cladosporium cladosporioides* were also common fungi on/in seeds, occurring usually with local or temporal peaks. Other fungi, including *Fusarium, Penicillium* and *Phoma* species, occurred sporadically.

Key words: *Amaranthus, A. cruentus, A. paniculatus, A. retroflexus,* seed-borne fungi

Introduction

Significant interest from farmers and consumers in the introduction and cultivation of new crops has been observed recently. A new crop is defined as a crop or crop product new to a certain area. *Amaranthus* can be included among them in Europe (Nalborczyk 1994). *Amaranthus* is a cosmopolitan genus of herbs, with 60 recognized species. It originates from South America. Although several species are often considered to be weeds, amaranths are valued around the world as leaf vegetables, food grains and ornamentals.

Amaranthus cruentus is the species that yields the nutritious staple amaranth grain. It is one of three *Amaranthus* species which can be cultivated as a grain source. In Central America it was in use as a food source as early as 4000 BC. Currently it is often grown and sold as a health food. It is an important crop for subsistence farmers in Africa.

Amaranthus retroflexus is a common tumble-weed (Rahban 1993, Michel et al. 1997). The plant was used for a multitude of food and medicinal purposes by many

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Native American groups. Currently it is often eaten as a vegetable in different parts of the world.

In Poland, there has been some research on *Amaranthus* spp., mainly on *A. cruentus*. Roszewski (1994), Songin and Sławiński (1999), and Szot (1999) studied *A. cruentus* from the point of view of its morphology, physiology, grain composition, methods of cultivation and importance and utilization in the food industry. Pusz (2005, 2008), and Pusz and Pląskowska (2008) studied its health in the temperate climate of Poland. Some reasearch was also done on the less common *Amaranthus* species, *A. hybridus* (Korbas 2006).

The objective of this study was to investigate the fungi on and within seeds of *A. cruentus, A. paniculatus* and *A. retroflexus* grown in Poland.

Materials and methods

Plants for mycological analyses were collected in 2003–2005 from *A. cruentus* (grown in Łosiów (50°51' N, 17°28' E) and Pawłowice (51°10' N, 17°12' E)), *A. paniculatus* (grown in Biskupin) and *A. retroflexus* (grown in Biskupin, Łosiów, Pawłowice and Swojec) at the seed-ripening stage. Each sample consisted of 30 plants. Seeds were harvested manually from panicle placed in cotton bags. Two hundred seeds were chosen randomly from each sample. One hundred seeds were surface-disinfected in sodium hypochlorite (1% available chlorine) for 30 s before being placed on 2% malt agar in Petri dishes. Another hundred seeds, with no surface disinfection, were placed on 2% malt agar in Petri dishes. After incubation for 14 days at 22°C, the plates were examined microscopically. Subcultures on potato dextrose agar (PDA) slants were made for preservation of cultures. Sporulating fungi were identified on the basis of their morphology according to the available literature. The frequency of a single taxon (in %) was calculated on the basis of its contribution to the total number of isolates from a treatment. Fungi recorded from non-disinfected seeds were considered to have grown, at least partly, from the surface of the seeds, and those recorded from surface-disinfected seeds were considered to have grown from inner tissue of the seed.

Results

Seeds of *A. cruentus, A. paniculatus* and *A. retroflexus* grown in Poland were colonized at least by 18 species of fungi (Tables 1, 2). Mycobiota recorded from surface-disinfected and non-disinfected seeds were similar qualitatively (consisting of the same range of species) and were different quantitatively (frequencies of individual species differed). Non-disinfected seeds were more intensively colonized by fungi than disinfected seeds, suggesting that a large proportion of infection was on the seed surface. Particularly large fungal communities were recorded on seeds of *A. cruentus* from Pawłowice and of *A. paniculatus*.

Table 1

Frequency of fungi isolated from surface-disinfected seeds of *Amaranthus* spp. (%)

Fungus	*Amaranthus cruentus*						*Amaranthus paniculatus*			*Amaranthus retroflexus*											
	Łosiów			Pawłowice			Biskupin			Biskupin			Łosiów			Pawłowice			Swojec		
	2003	2004	2005	2003	2004	2005	2003	2004	2005	2003	2004	2005	2003	2004	2005	2003	2004	2005	2003	2004	2005
Alternaria alternata	73.21	72.09	43.24	68.75	78.38	56.25	11.11	70.73	55.56	80.00	72.22	54.55	75.00	85.29	7.41	58.33	31.58	25.58	66.67	37.93	36.96
Arthrinium phaeospermum	–	–	–	–	–	0.89	11.11	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aspergillus niger	–	–	–	–	–	1.79	11.11	9.76	14.81	–	–	4.55	–	–	–	–	–	–	–	–	2.17
Cladosporium cladosporioides	–	2.33	16.22	9.38	2.70	10.71	11.11	7.32	11.11	6.67	5.56	4.55	–	2.94	37.04	25.00	55.26	34.88	–	41.38	23.91
Epicoccum nigrum	8.93	9.30	22.97	15.63	2.70	27.68	11.11	2.44	3.70	13.33	–	31.82	25.00	–	22.22	16.67	–	27.91	–	–	26.09
Fusarium avenaceum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3.45	2.17
Fusarium culmorum	–	2.33	1.35	–	–	0.89	–	–	–	–	–	–	–	–	3.70	–	2.63	2.33	–	6.90	–
Fusarium equiseti	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3.70	–	–	–	22.22	3.45	–
Penicillium notatum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.33	–	–	–
Penicillium waksmani	–	–	–	–	–	–	–	–	–	–	–	4.55	–	–	1.85	–	–	2.33	–	–	–
Periconia minutissima	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.85	–	–	–	–	–	–
Phoma eupyrena	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.33	–	–	–
Phoma exigua	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.85	–	–	–	–	3.45	2.17
Rhizopus arrhizus	17.86	4.65	–	–	5.41	–	–	–	–	–	–	–	–	–	1.85	–	–	–	11.11	3.45	–
Trichoderma harzianum	–	–	1.35	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ulocladium botrytis	–	–	4.05	6.25	–	–	–	–	–	–	–	–	–	–	–	–	–	2.33	–	–	–
Non-sporulating fungi	–	9.30	10.81	–	10.81	1.79	44.44	9.76	14.81	–	22.22	–	–	11.76	18.52	–	10.53	–	–	–	6.52

Table 2

Frequency of fungi isolated from non-disinfected seeds of *Amaranthus* spp. (%)

Fungus	*Amaranthus cruentus*						*Amaranthus paniculatus*			*Amaranthus retroflexus*											
	Łosiów			Pawłowice			Biskupin			Biskupin			Łosiów			Pawłowice			Swojec		
	2003	2004	2005	2003	2004	2005	2003	2004	2005	2003	2004	2005	2003	2004	2005	2003	2004	2005	2003	2004	2005
Alternaria alternata	83.93	84.31	54.64	79.49	81.58	58.29	62.61	64.14	73.50	79.12	76.04	75.00	82.56	80.00	20.78	57.73	48.94	44.44	61.15	70.75	52.43
Aspergillus niger	–	–	–	–	2.63	6.86	–	2.07	1.71	–	–	–	–	–	–	12.37	–	–	–	8.49	2.91
Cladosporium cladosporioides	–	–	10.31	–	1.75	8.00	20.00	24.14	12.82	–	4.17	5.68	–	1.90	11.69	1.03	25.53	18.06	–	–	11.65
Epicoccum nigrum	12.50	7.84	21.65	8.55	7.02	21.71	10.43	5.52	6.84	13.19	13.54	7.95	16.28	7.62	27.27	12.37	6.38	12.50	25.90	16.04	13.59
Fusarium avenaceum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5.19	–	–	–	–	–	–
Fusarium culmorum	–	–	–	–	0.88	1.14	–	2.07	2.56	–	–	–	–	–	5.19	1.03	2.13	4.17	–	–	0.97
Fusarium equiseti	–	–	1.03	–	–	–	–	–	–	–	–	–	–	–	1.30	1.03	–	–	5.04	–	1.94
Mucor hiemalis	–	–	–	2.56	–	–	–	–	–	–	–	–	–	–	1.30	1.03	–	1.39	–	–	2.91
Penicillium notatum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.60	–	–	1.39	–	–	–
Penicillium waksmani	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.60	–	–	1.39	–	–	–
Phoma eupyrena	–	–	–	–	–	0.57	–	–	–	–	–	–	–	–	–	–	–	1.39	–	0.94	–
Rhizopus arrhizus	–	6.86	3.09	–	6.14	1.14	–	2.07	2.56	–	–	4.55	–	6.67	1.30	1.03	–	4.17	–	–	6.80
Stemphylium vesicarium	–	–	–	–	–	–	0.87	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ulocladium botrytis	–	0.98	3.09	–	–	1.71	–	–	–	–	–	–	–	1.90	1.30	–	2.13	1.39	–	0.94	0.97
Non-sporulating fungi	3.57	–	6.19	9.40	–	0.57	6.09	–	–	7.69	6.25	5.68	1.16	1.90	22.08	12.37	12.77	11.11	7.91	2.83	5.83

Alternaria alternata was the most common species. Its frequency on the surface and inside of seeds was similar and amounted to 57–84% of isolations. Its smallest frequencies were recorded on seeds of *A. paniculatus* (11%) and *A. retroflexus* (7.5%) in 2005. *Epicoccum nigrum* and *Cladosporium cladosporioides* were other common fungi on/in seeds, occurring usually with local or temporal peaks. The frequencies of *E. nigrum* amounted to 5–27% on non-disinfected seeds and up to 32% as internal infections. The frequencies of *C. cladosporioides* amounted to 26% on non-disinfected seeds and up to 56% as internal infections. *Cladosporium cladosporioides* was particularly common on seeds of *A. paniculatus* and in seeds of *A. retroflexus*. Other fungi, including *Fusarium, Penicillium* and *Phoma* species, occurred sporadically.

Discussion

Seeds of *A. cruentus, A. paniculatus* and *A. retroflexus* were strongly colonized by *A. alternata*. Its common occurrence on surface-sterilized seed indicates the ability of the fungus to penetrate the internal tissues of seeds. The high levels of internal infection may indicate that *A. alternata* was not a random contaminant, but that its presence resulted from a direct colonization of flowers or seeds.

The results presented agree with observations of Noelting et al. (2004), who reported that *Alternaria* species, including *A. alternata*, are the most common colonizers of *A. cruentus* seeds. The tendency of *Alternaria* species to infect seeds internally was observed also in other plants, including oilseed rape (Maude and Humpherson-Jones 1980, Jajor 2006).

In cereals, the presence of *A. alternata* in stored grain does not always decrease its germination capacity (Narkiewicz-Jodko 1986, 1998, Narkiewicz-Jodko and Gil 1997). A more important indicator of lower grain quality is the occurrence of *Fusarium* species (Sadowski et al. 2007). *Fusarium* species occurred only sporadically on/in seeds of *Amaranthus*. Its frequency varied according to the species of *Amaranthus*, location and year.

Conclusions

1. *Alternaria alternata* was the most common fungus colonizing seeds of *A. cruentus, A. paniculatus* and *A. retroflexus*.
2. *Epicoccum nigrum* and *Cladosporium cladosporioides* were also common fungi occurring usually with local or temporal peaks.
3. Other fungi, including *Fusarium, Penicillium* and *Phoma* species, occurred sporadically.

Streszczenie

GRZYBY ZASIEDLAJĄCE NASIONA ROŚLIN RODZAJU *AMARANTHUS*

Nasiona *Amaranthus cruentus, A. paniculatus* i *A. retroflexus* zebrane z plantacji zlokalizowanych w Polsce południowo-zachodniej były zasiedlone przez 18 gatunków grzybów. Najczęściej wyosobnianym gatunkiem był *Alternaria alternata*. W niektórych przypadkach stanowił on 85% wszystkich izolowanych kolonii. Występowanie tego gatunku w tak dużej ilości może świadczyć o infekcji już w fazie kwitnienia. Często izolowano również *Epicoccum nigrum* i *Cladosporium cladosporioides*. Grzyby rodzajów *Fusarium, Penicillium* i *Phoma* były izolowane sporadycznie.

Literature

Jajor E., 2006: Zasiedlenie przez grzyby nasion odmian populacyjnych i mieszańcowych rzepaku pochodzących ze zbioru 2004. Progr. Plant Prot. / Post. Ochr. Rośl. 46, 2: 572–575.

Korbas M., 2006: Choroby *Amaranthus hybridus* L. i możliwości ich zwalczania. Progr. Plant Prot. / Post. Ochr. Rośl. 46, 1: 127–131.

Maude R.B., Humpherson-Jones F.M., 1980: Studies on the seed-borne phases of dark leaf spot (*Alternaria brassicicola*) and grey leaf spot (*Alternaria brassicae*) of brassicas. Ann. Appl. Biol. 95: 311–319.

Michel A., Albrecht H., Ulrich H., Hurle K., 1997: RAPD-PCR based differentiation of herbicide resistant *Amaranthus retroflexus*. In: European Weed Research Society 10th Symposium Poznań 10–12.06.1997. Poznań: 195–200.

Nalborczyk E., 1994: Biologia amaranthusa oraz perspektywy jego uprawy i wykorzystania w Polsce. In: Nowe rośliny uprawne: *Amaranthus*. Ed. E. Nalborczyk. Wyd. SGGW, Warszawa: 8–29.

Nalborczyk E., 1999: Rośliny alternatywne rolnictwa XXI wieku i perspektywy ich wykorzystania. Zesz. Probl. Post. Nauk Roln. 468: 17–30.

Narkiewicz-Jodko M., 1986: Wartość siewna przechowywanego ziarna trzech zbóż w aspekcie fitopatologicznym. Zesz. Nauk. AR Wroc. Rozpr. 55.

Narkiewicz-Jodko M., 1998: Zdrowotność ziarna zbóż jako wskaźnik jego jakości. Zesz. Nauk. AR Wroc. 328, Technol. Żywn. 12: 85–93.

Narkiewicz-Jodko M., Gil Z., 1997: Wpływ przedplonu na zdrowotność i jakość pszenicy ozimej. Plant Breed. Seed Sci. 41, 1: 83–88.

Noelting M.C., Sandoval M.C., Abbiati N.N., 2004: Determinación de microorganismos fúngicos en semillas de amaranto (*Amaranthus* spp.) mediante diferentes métodos de análisis. Rev. Peru. Biol. 11, 2: 1–15.

Pusz W., 2005: Mikoherbicydy, czyli możliwości wykorzystania grzybów do ograniczania zachwaszczenia. Ochr. Rośl. 11: 30–32.

Pusz W., 2008: Occurrence of dark spots on stems of *Amaranthus* species. Phytopathol. Pol. 48: 53–57.

Pusz W., Pląskowska E., 2008: *Phomopsis amaranthicola* – nowy patogen szarłatu uprawnego (*Amaranthus cruentus*). Zesz. Probl. Post. Nauk Roln. 529: 175–182.

Rahban B.A., 1993: Zwalczanie szarłatu szorstkiego w uprawie buraka cukrowego. Ochr. Rośl. 6: 8–9.

Roszewski R., 1994: Uprawa szarłatu (*Amaranthus* spp.) na nasiona. In: *Amaranthus* – perspektywy uprawy i wykorzystania. Ed. E. Nalborczyk. Wyd. SGGW, Warszawa: 26–34.

Sadowski Cz., Lenc L., Wyczling D., 2007: Grzyby z rodzaju *Fusarium* występujące w ziarnie wybranych odmian pszenicy uprawianej w rejonie Żuław. Progr. Plant Prot. / Post. Ochr. Rośl. 47, 2: 306–309.

Songin H., Sławiński K., 1999: Wpływ sposobu zbioru na plonowanie i wigor nasion szarłatu uprawnego (*Amaranthus* spp.). Zesz. Probl. Post. Nauk Roln. 468: 429–433.

Szot B., 1999: Właściwości agrofizyczne amarantusa (*Amaranthus cruentus* L.). Acta Agrophys. 18.

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