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MYCOLOGICAL AIR CONTAMINATION IN A SEED COMPANY PROCESSING ROOM AS A RESULT OF SEED PROCESSING[1]

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Abstract

The concentration of fungal spores in the air of a seed company processing room was determined using Lanzoni volumetric trap and by gravimetric method. The analyses were performed before and during onion, dill and carrot seed processing. Moreover, mycological analysis was performed for cleaned seeds and plant residues and other impurities obtained in the process. It was found that carrot seed cleaning and separating resulted in considerable increase in fungal spore concentration in the air. Fungal spores in the air can adversely affect human's health by causing allergic diseases and could be a source of mycotoxins. Spores of fungi pathogenic to plants were present in the air, too.

Key words: air contamination, fungi, seed health, seed processing, spores

Introduction

Microbiological contamination of air, water and soil significantly influences quality of human's life and their health status. A large number of microorganisms and their fragments and other biological particles, including fungal spores, have been passively transferred to the atmosphere, creating a bio-aerosol. Fungi, whose spores are most often present in the air, belong to *Basidiomycota*, e.g.: *Coprinus*, *Ganoderma*, *Tilletia*, etc., *Zygomycota*, e.g.: *Mucor* and *Rhizopus* and to mitosporic fungi – previously Fungi imperfecti, e.g.: *Alternaria*, *Cladosporium*, *Epicoccum*, *Drech-*

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slera, *Stemphylium*, *Aspergillus*, *Penicillium*, *Fusarium*, etc. They are known to produce allergens and may elicit allergic reactions (Horner et al. 1995). Many species pathogenic to plants occur in these groups, too. A lot of them are seed-borne (Richardson 1990). Apart from pathogens seeds are associated with broad range of saprothrophic microorganisms, also belonging to the groups mentioned above.

Harvesting and threshing cereal grains, flax, herbs including thyme, chamomile, sage, valerian roots and other crops is accompanied by a release of organic dust and bio-aerosol, including fungal spores, which can caused allergic and immuno-toxic reactions in humans (Skórska et al. 1998, Krysińska-Traczyk et al. 2001, Golec et al. 2004, Nordby et al. 2004, Skórska et al. 2005 a, b). The list of diseases caused in humans by fungal allergens includes asthma, chronic bronchitis, allergic alveolitis, rhinitis and conjunctivitis (Krysińska-Traczyk et al. 2003). Sensitivity to particular allergens can appear in a healthy individual as a result of long-term stay in an environment polluted with fungi, mainly with their spores. The seemingly insignificant contact with microorganisms can be followed by a development of work-related diseases, e.g. "teapicker's disease" (Takahashi 1997).

Beside allergenic properties, some seed-borne fungi are capable of producing mycotoxins. Aflatoxins produced mostly by *Aspergillus flavus* and *A. parasiticus*, ochratoxin A produced mainly by *Penicillium verrucosum* and *A. ochraceus*, nivalenol, deoxynivalenol and moniliformin produced by *Fusarium* spp., and alternariol and tenuazonic acid produced by *Alternaria* spp. are considered the most dangerous for humans and animals (Skaug et al. 2000, Krysińska-Traczyk et al. 2003, Nordby et al. 2004, Straumfors-Halstensen et al. 2004, Solfrizzo et al. 2005).

Concentration of the fungal spores in the air has been changing depending on place, season, time of a day, weather and human's activity. In general, *Alternaria*, *Cladosporium*, *Penicillium* and *Aspergillus* are the dominating species in the outdoor air. Species of *Rhizopus*, *Penicillium*, *Aspergillus*, *Alternaria* and *Cladosporium* are the most often recorded ones in the indoor air (Horner et al. 1995, Takahashi 1997).

Assuming that spores present in the air can be a source of human allergies and secondary infections of seeds, the experiments were conducted to study the influence of industrial seed cleaning and separating on fungal spore air contamination in the processing room of a seed company.

Materials and methods

Samples of the air, seeds and impurities were collected in a processing room of a seed company in 2007, before, during and after processing of carrot (*Daucus carota*) cv. A seeds, dill (*Anethum graveolens*) cv. B seeds and onion (*Allium cepa*) cvs. C and D seeds. The seeds were cleaned and separated using Petkus Super K541 cleaning machine.

Analysis of the air

The air samples were taken 1.5 m away from the machine, 1.4 m above the ground, using volumetric and gravimetric methods, each time at the same place.

Volumetric method. The samples were collected for 5 min at the flow rate of 10 l/min before seed cleaning and separating, and after 15 min from the start of seed processing, by means of the Lanzoni volumetric trap. Bio-aerosol sucked in along with the air was deposited on a microscopic slide covered with a mixture of liquid paraffin, vaseline and adhesive toluene. The slide was moving with a constant speed of 2 mm per hour. The collected material was covered with glass and protected by a transparent nail polish. Deposit corresponded to contaminations present in the 0.05 m^3 of air. Fungal spores were counted and identified on the basis of their morphology. The results are presented as the number of spores per 1 m^3 of the air.

Gravimetric method. Ten 9 cm diameter Petri dishes filled with approximately 15 ml of solid potato dextrose agar medium (PDA, Scherlau Chemie, Spain) supplemented with 100 ppm streptomycin, were exposed for 5 min for free spore sedimentation before seed processing and 15 min after the process had started. Afterwards the dishes were placed for seven days at 20°C under 12 h alternating cycles of near ultraviolet (NUV) light and darkness. The fungal colonies were counted and identified using stereomicroscope and compound microscope. The results are presented as number of colony forming units (CFUs) per 1 m^3 of the air.

Mycological analysis of seeds and impurities

The cleaned seeds and three fractions of impurities, i.e. the smallest impurities, impurities from upper sieves and impurities from bottom sieves were collected after processing. 1 g of each sample was suspended in 100 ml of a sterile solution of 0.01% Tween 80. After 5 min of vigorous shaking, 0.1 ml of each suspension was uniformly distributed on solid PDA medium amended with 100 ppm streptomycin, using Drigalski spatula. There were five replicates for cleaned seeds and each fraction of impurities. Seeds were incubated for 10 days at 20°C under 12 h alternating cycles of NUV light and darkness. The fungal colonies were counted and identified using stereomicroscope and compound microscope. The results are presented as number of CFUs per 1 g of cleaned seeds and each fraction of impurities, respectively.

Moreover, the standard seed health analysis was performed on 200 seeds from each sample. Deep-freeze blotter method was applied for carrot and dill seeds, whereas agar test for onion seeds. In the blotter test seeds were plated on six layers of moistened blotters placed in 9 cm diameter Petri dishes, 20 seeds per dish. The seeds were incubated in darkness at 20°C for three days, at –20°C for 24 h and then for eight days at 20°C under 12 h alternating cycles of NUV light and darkness. In the agar test seeds were plated in 9 cm diameter Petri dishes, on PDA medium supplemented with streptomycin, 10 seeds per dish. Seeds were incubated for 10 days at 20°C under 12 h alternating cycles of NUV light and darkness. Afterwards, they

were examined for the presence of fungi using stereomicroscope and compound microscope (Machado et al. 2002, Mathur and Kongsdal 2003). The results are presented as the percentage of seeds colonized with each fungal species or genus.

Statistical analysis

Results obtained with the gravimetric method were evaluated with analysis of variance followed by Duncan's multiple range test (Kala 2002).

Results

The environment in the company processing room proved highly contaminated with fungal spores. Regardless of conducted works, very high concentration of *Alternaria* and *Cladosporium* spp. spores was found in the air, as measured with the volumetric method (Table 1). The number of *Alternaria* spp. spores increased in the air markedly during processing of carrot and onion seeds, whereas the higher number of *Cladosporium* spp. spores was observed in case of processing of carrot, dill and onion cv. D seeds. Cleaning and separating carrot seeds resulted also in an increased concentration of *Botrytis* spp., *Epicoccum purpurascens* and *Stemphylium* spp. spores in the air. Seed processing increased air contamination with *Botrytis* spp. spores in the case of both onion cultivars and with *E. purpurascens* in the case of cv. C. The fungi that occurred at the value below 40 spores per 1 m^3 were not presented in Table 1.

The numbers of CFUs per 1 m^3 of the air obtained from deposition in the gravimetric method were relatively high considering the spore concentrations in the air detected by the volumetric method. However, the proportions between values before and during seed processing were stable. The fungi which occurred both before and during seed processing at the value below 18 CFUs per 1 m^3 of the air, as measured with the gravimetric method, were not presented in Table 2. The differences in the numbers of fungal spores in the air, measured with the volumetric method, and CFUs in the air, measured with the gravimetric method, before and during seed cleaning and separating were observed mainly in the case of carrot (Tables 1 and 2). Moreover, a significant increase in CFUs of *Cladosporium* spp. was recorded, when onion cv. C seeds were cleaned and separated (Table 2).

All examined seed lots fractions of the smallest impurities were infested with fungi to the highest level (Table 3). The fungi which occurred at the value below 200 CFUs per 1 g were not presented in the Table.

Standard seed health test showed high infestation of carrot seeds with *Alternaria alternata*, *Cladosporium* spp., *Epicoccum purpurascens*, *Fusarium* spp., and *Gonatobotrys simplex*. Dill seeds were colonized mostly with *A. alternata*, *G. simplex* and *Trichothecium roseum*. Onion seeds were infested mainly with *A. alternata*, *Cladosporium* spp., *E. purpurascens* and *Penicillium* spp. (Table 4).

Table 1

Fungi in the air of the processing room of a seed company analysed with the Lanzoni volumetric trap

Fungus	Spores per 1 m^3 of the air	
	before seed processing	during seed processing
Carrot cv. A		
Acremoniella atra	0	40
Alternaria alternata	1 740	5 600
Alternaria radicina	60	40
Aspergillus sp.	40	0
Bipolaris sp.	60	0
Botrytis spp.	20	120
Cladosporium spp.	1 260	4 800
Dendryphion sp.	60	0
Drechslera sp.	40	0
Epicoccum purpurascens	0	100
Fusarium spp.	40	20
Melanomma pulvispyrius	40	0
Periconia sp.	60	0
Stemphylium spp.	140	780
Dill cv. B		
Alternaria alternata	220	160
Cladosporium spp.	420	540
Onion cv. C		
Alternaria alternata	380	620
Botrytis spp.	20	60
Cladosporium spp.	2 100	1 900
Epicoccum purpurascens	0	40
Stemphylium spp.	60	0
Onion cv. D		
Alternaria alternata	720	920
Cladosporium spp.	720	2 620
Botrytis spp.	0	40

Discussion

Alternaria, Aspergillus, Cladosporium and *Penicillium* which were dominating taxa found in the air of the seed company processing room are particularly strongly associated with allergic respiratory diseases, especially asthma. However, other fungi, present in the air, although less frequently, e.g. *Botrytis*, *Epicoccum*, *Fusarium*

Table 2

Fungi in the air of the processing room of a seed company analysed with the gravimetric method

Fungus	CFUs per 1 m^3 of the air	
	before seed processing	during seed processing
Carrot cv. A		
Alternaria alternata	400 a	10 273 b
Alternaria tenuissima	91 a	145 a
Aspergillus flavus	18 a	255 b
Cladosporium spp.	273 a	12 745 b
Epicoccum purpurascens	0 a	364 b
Fusarium spp.	18 a	509 b
Penicillium spp.	91 a	836 b
Dill cv. B		
Alternaria alternata	782 a	745 a
Aspergillus sp.	91 a	55 a
Cladosporium spp.	345 a	455 a
Gliocladium roseum	91 a	109 a
Penicillium spp.	582 a	327 a
Onion cv. C		
Alternaria alternata	127 a	127 a
Alternaria tenuissima	36 a	200 a
Aspergillus flavus	18 a	109 a
Aspergillus spp.	127 a	109 a
Botrytis spp.	55 a	55 a
Cladosporium spp.	218 a	727 b
Penicillium spp.	109 a	181 a
Onion cv. D		
Alternaria alternata	1 800 a	1 091 a
Cladosporium spp.	818 a	709 a
Fusarium spp.	55 a	91 a
Penicillium spp.	800 a	455 a

Means in the same row followed by the same letter are not significantly different at $\alpha = 0.05$ level according to Duncan's multiple range test.

and *Stemphylium* spp. are also important aeroallergens (Horner et al. 1995). Some of them, e.g. *Alternaria*, *Botrytis* and *Fusarium* spp., can also cause plant diseases and are known to be seed transmitted. That is why their presence in the air of seed company processing room can be considered a potential risk of seed secondary infection. Furthermore, the occurrence of *Aspergillus* and *Penicillium* spp. in the air and, in consequence, on the seeds, may influence seed health during storage. Infestation of seed with the fungi may result in loss of viability, increase in free fatty ac-

Table 3

Fungi in seed lot fractions after processing (CFU/g)

Fungus	The smallest impurities	Impurities from upper sieves	Impurities from bottom sieves	Cleaned seeds
Carrot cv. A				
Acremoniella atra	2 000	–	1 200	400
Alternaria alternata	25 000	–	7 600	7 800
Cladosporium spp.	89 600	–	11 200	15 200
Epicoccum purpurascens	1 000	–	9 400	4 400
Fusarium spp.	130 000	–	18 000	50 200
Penicillium spp.	0	–	400	1 400
Dill cv. B				
Acremoniella atra	0	0	200	5 200
Alternaria alternata	10 000	4 200	6 800	6 200
Aureobasidium pullulans	1 400	0	0	0
Cladosporium spp.	1 800	400	1 000	2 200
Penicillium spp.	3 400	400	4 000	200
Phoma sp.	8 000	400	200	0
Onion cv. C				
Acremoniella atra	122 000	3 600	66 000	33 800
Alternaria alternata	800	3 800	5 200	1 000
Aureobasidium pullulans	0	0	0	1 400
Cladosporium spp.	9 000	2 600	4 000	1 400
Epicoccum purpurascens	14 000	3 000	2 600	1 800
Fusarium spp.	32 400	28 200	25 200	13 200
Mucor sp.	0	200	4 600	0
Phoma sp.	1 000	0	0	0
Onion cv. D				
Cladosporium spp.	0	–	1 000	0

"–" – not obtained.

ids, decrease in nonreducing sugars, development of musty odours and discoloration (Justice and Bass 1978).

Processing each seed lot favoured releasing spores of particular fungi. Regardless of other factors, presumably the plant species, and in consequence different way of seed formation and seed surface structures, had a close relationship with the amount and composition of the fungal spores present in the atmosphere. Rough surface of carrot seed seems to be a good niche for many free spores, which can be easily released during technological processes. Spores, being small particles, were found included in large numbers in the fraction of the smallest impurities. Hence, appropriate precautions should be taken during transportation and recycling of the residues, to prevent further release of spores.

Table 4

Seeds infested by fungi after processing analysed with the standard seed health tests (%)

Fungus	Carrot cv. A	Dill cv. B	Onion cv. C	Onion cv. D
Acremoniella atra	2.5	0	0	0
Alternaria alternata	99.5	99.5	56.5	15.5
Alternaria dauci	1.5	1.0	0	0
Alternaria radicina	1.5	0	0	0
Alternaria tenuissima	0	2.0	0	0
Aspergillus flavus	0	0	1.5	0.5
Aspergillus spp.	0	0	1.0	4.5
Aureobasidium pullulans	0	0	1.5	1.0
Botrytis aclada	0	0	6.0	0
Botrytis cinerea	0.5	10.0	9.0	2.0
Cephalosporium spp.	1.5	0	0	0
Cladosporium spp.	19.0	6.0	25.0	17.5
Drechslera spp.	0	1.5	0	0
Epicoccum purpurascens	24.0	10.5	22.0	0
Fusarium spp.	38.5	5.0	5.5	1.5
Gonatobotrys simplex	27.5	67.5	2.0	0
Papularia sp.	0	0	0.5	0
Penicillium spp.	2.0	0	37.0	18.0
Pleospora herbarum	0	0	0	0.5
Rhizopus nigricans	0	1.0	0	0
Stemphylium spp.	5.0	5.5	0	0
Trichothecium roseum	0	62.5	1.5	3.0
Ulocladium sp.	0	0.5	0	0

Undoubtedly, *Alternaria* and *Cladosporium* spores were predominant, regardless of the seed lot and technique of measurement. For these fungi, the threshold concentrations for evoking symptoms of allergy in humans were 100 and 3000 spores per 1 m^3 of the air for *Alternaria* and *Cladosporium* spp., respectively (Gravesen 1979). The threshold level for evoking allergic symptoms for *Alternaria* spp. was exceeded always during the experiment. However, only during processing carrot seeds, the number of *Cladosporium* spp. spores per 1 m^3 of the air exceeded 3000. It was found that the concentration could have been multiplied during agricultural procedures, such as grain harvesting, threshing, storage and grinding, creating a health hazard to farm workers (Savino and Caretta 1992, Mediavilla Molina et al. 1996, Atluri and Murthy 2002). Beside these two "high risk" genera, spores of various species of *Aspergillus*, *Botrytis*, *Penicillium*, and *Stemphylium* could adversely

affect the staff of the seed company. Special attention should be paid to the presence of *Aspergillus flavus*, because of its particularly dangerous effects on vertebrates.

The methods applied in the study gave different information on the presence of fungi in the air and seed lots. Considerable seed infestation with fungi detected with the standard seed health test determined high spore release, but it was not a rule. For instance, 99.5% of carrot and dill seeds were infested with *A. alternata*, but only in the case of carrot it was connected with significant release of spores during cleaning and separating. It can suggest that most of the fungi were connected with the seed tissues, and only some of them were seeds contaminants.

The Lanzoni volumetric trap method could not be evaluated for its repeatability and sensitivity because only a single sampler was operated. However, the Lanzoni spore trap sampler does provide a possibility to determine total numbers of airborne spores, eliminating the reliance on spore viability for detection. Determination of total spore number is important because allergic reaction may be caused by the presence of fungal antigens and may not be connected to the viability of the fungus. Generally, the numbers of CFUs per 1 m^3 of the air obtained from deposition in the gravimetric method corresponded closely to spore concentrations recorded with the volumetric method. From a seed pathologist's point of view, the gravimetric method, which determined only viable spores, seems to be more useful in forecasting potential secondary seed infection.

For both reasons, i.e. human and plant health, the use of efficient aspiration systems and respirators is particularly recommended.

Streszczenie

ZANIECZYSZCZENIE MIKOLOGICZNE POWIETRZA W HALI PRODUKCYJNEJ PRZEDSIĘBIORSTWA NASIENNEGO JAKO SKUTEK USZLACHETNIANIA NASION

Zarodniki grzybów w powietrzu mogą niekorzystnie wpływać na zdrowie człowieka, będąc przyczyną chorób alergicznych oraz źródłem mikotoksyn. Wśród nich znajdują się również zarodniki gatunków chorobotwórczych dla roślin. Stężenie zarodników występujących w powietrzu hali przedsiębiorstwa nasiennego przed uszlachetnianiem i w trakcie uszlachetniania nasion marchwi, kopru i cebuli określano za pomocą aparatu wolumetrycznego Lanzoniego oraz metody grawimetrycznej. Analizie mikologicznej poddawano także oczyszczone nasiona oraz odpady powstałe podczas ich czyszczenia i sortowania. Powietrze w hali przerobowej przedsiębiorstwa było w znacznym stopniu zanieczyszczone zarodnikami grzybów. Duża była zwłaszcza koncentracja zarodników grzybów dwóch głównych rodzajów wywołujących alergie: *Alternaria* i *Cladosporium*. Uszlachetnianie nasion marchwi w największym stopniu wpływało na zwiększenie stężenia zarodników grzybów w powietrzu. Frakcja najmniejszych zanieczyszczeń, otrzymanych podczas przerobu nasion, zawierała najwięcej zarodników grzybów.

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