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Seed-borne fungi of *Ornithopus sativus* during a twenty year storage

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Subject to analysis were seed-borne fungi of serradella during a long term storage. The agents causing a significant reduction of seed-borne fungi were: the storage conditions and time (very low seed moisture, approx. 5.5%, no oxygen).

Key words: fungi, serradella seeds, survival, storage.

INTRODUCTION

Among many examined seeds the leguminous seeds are considered long-lived (W i l t o n et al. 1978). Aside from high initial germinability, one of the essential criteria in selecting batches of seeds intended for a long term storage is the assessment of their health.

On the one hand, adequate storage conditions make it possible to retain high sowing value and, on the other, they are able to limit the growth of the fungi settled.

The aim of the study was to observe the changes in seed-borne fungi communities of serradella during a 20 year storage and to determine the germinability of the examined seeds.

MATERIALS AND METHODS

The experimental materials were three batches of serradella seeds (Bydgoska cultivar, original) harvested in 1973. The seeds dried by means natural desorption to the moisture of about 5.5% were stored in tight, glass vessels in a store room (5-29°C) for twenty years.

Subject to examination were:

1. the composition of species and number of seed-borne fungi isolated from serradella by means of the Ulster method and the adjusted Ulster method (Narkiewicz-Jodko and Schneider 1983).

2. germination capacity of the seeds according to the Polish Standard requirements (PN-69/R-65-950, PN-79/R-65-950).

RESULTS

The mycological assessment revealed significant changes in the fungi communities settling the surface of the serradella seeds (Tab. 1). The composition of species and the number of fungi settling the fresh seeds ('0' — initial analysis) were greatly varieable. 27 species were identified, *Alternaria alternata* being by far the most dominant species, amounting to about 80% of the isolates. With the passage of time a significant reduction of the whole fungi community was noted, *Alternaria alternata* in particular. The greatest reduction of fungi isolates (approx. 50%) was noted between the fifth and the tenth years of storage. After the first three years of storage no fungi from *Fusarium* species were isolated.

The species isolated after the 20-year storage included: *Alternaria alternata*, *Absidia corymbifera*, *Aspergillus chevalieri*, *A. repens*, *Cladosporium cladosporioides*, *Mucor racemosus*, *Penicillium cyclopium*, *P. notatum* and *Sordaria fimicola*.

As a result of the mycological analyses of disinfected seeds it was established that the fungal communities settling the endophytic tissues of the seeds both before and during the storage, were distinctly poorer (Tab. 2). Among the isolated species *Alternaria alternata* was dominant, especially in the fresh seeds ('0' — initial analysis). After a five year storage a considerable reduction of the fungi was noted. However, in the final phase of the storage (after 20 years) single isolates were found, namely: *Alternaria alternata*, *Cladosporium cladosporioides* and non-sporulating colonies.

The analysis of seed viability revealed very high level of their germinalibity (95-98%). After 10 years the seeds of the two batches (753 and 754) germinated at 89 and 91% (class I according to the Polish Standard), after 15 years, though, only the seeds from one batch (753) had a good viability — 77% (class II). In the final phase of the storage (after 20 years) the seeds from the three batches germinated within the ranges from 27% to 55%. The seeds from the batches which were the most intensely infested by fungi from *Aspergillus* and *Penicillium* species (754 and 870) germinated at the lowest level.

DISCUSSION

With the passage of time a gradual reduction of seed-borne fungi of serradella was detected. The greatest reduction of fungi isolated from the seeds was found between the fifth and the tenth year of the storage.

The gradual decrease of the number of fungi isolated from papilionaceous seeds under storage conditions was discerned by many authors, e.g. Grzelak (1964), Truszkowska et al. (1970), Narkiewicz–Jodko (1973) and Narkiewicz–Jodko and Schneider (1989).

The survival of fungi settling the seeds is relative to time storage conditions (temperature and moisture) and location of the fungi in the seeds (Neergaard 1979; Welty et al. 1987; Huang et al. 1994). On classifying the seed-borne fungi of serradella into short and long-lived (as suggested by Pelhâte 1968), it was found that the group of short-lived fungi included: *Fusarium oxysporum*, *F. solani*, *Papularia rosea* (up to 2 years), *Sclerotinia sclerotiorum* (to 5 years). This opinion is in agreement with the results by Czyżewska (1993).

In the long-lived fungi (according to Neergaard 1979) these species were included which survived in the seeds for over 10 years. They were the following: *Absidia corymbifera*, *Acremoniella atra*, *Alternaria alternata*, *Aspergillus chevalieri*, *A. repens*, *Aureobasidium pullulans*, *Chaetomium funicola*, *Cladosporium cladosporioides*, *Mucor racemosus*, *Penicillium cyclopium*, *P. notatum*, *Phoma trifolii*, *Rhizopus nigricans*, *Sordaria fimicola* and *Stemphylium botryosum*.

The fungi settling stored seeds were examined by many authors. However, the storage period of the sowing material was definitely shorter than in the present experiment and, besides, the storage method was quite different (Rolston et al. 1986; Hewett 1987; Siddiqui and Mathur 1988; Maholay 1994).

The storage conditions unfavourable for the development of fungi (low seed moisture — 5.5%, no oxygen) as well as a very long storage term failed to eliminate *Alternaria alternata* and *Stemphylium botryosum*. The previous study by the author (Narkiewicz–Jodko 1973) indicates that *Alternaria alternata* and *Stemphylium illicis* caused serradella seedling decay.

The gradual degradation of the seed germination was presumably caused by long storage term. However, the greatest degradation of germinability was noted in the seeds from batches 754 and 870, which were most intensely infested by storage fungi of *Aspergillus* and *Penicillium* species. This opinion corroborates the observations by Gupta et al. (1993) who have previously identified the effect of storage fungi on the viability of soy seed.

Table 1
Seed-borne fungi of serradella (harvested in 1973) during a 20 year storage

Fungi	Seed batches (numbers)														
	753					754					870				
	Years of storage														
	0	5	10	15	20	0	5	10	15	20	0	5	10	15	20
Absidia corymbifera (Cohn) Sacc.		1	4	4	1		2	2	2		1	1	6		
Acremoniela atra (Corda) Sacc.								1			1	1		3	
Alternaria alternata (Fr.) Keissler	76	56	11	5	4	83	65	23	3	4	69	38	9	3	2
Arthrinium arundinis (Corda) M. B. Ellis	3	5				1									
Aspergillus chevalieri (Mangin) Thom and Church.								5	4	4	1		5	8	12
Aspergillus repens de Bary											1	1	5	10	10
Aureobasidium pullulans (de Bary) Arnaud	7	8	6	3		2	5	5	2		4	7	4	3	
Chaetomium funicolum Cooke			2	2					1					2	
Cladosporium cladosporioides (Fres.) de Vries					2	2	1			1	3	1			
Fusarium oxsysporum (Schlecht.)						2					2				
Fusarium solani (Mart.) Sacc.						1									
Gonatobotrys simplex Corda						1					1				
Mucor racemosus Fresenius	4	4	11	5	1	1			3		1				
Mucor spinosus van Tieghem		4						1			1	1			
Papularia rosea Gragben et Kuznetz	4					2									
Penicillium chrysogenum Thom	2	2					2				4	3			
Penicillium corymbiferum Westling	3	5	4			2	2	2			4	2			
Penicillium cyclopium Westling	2	5	4			2	2	3			4	4	3	5	
Penicillium notatum Westling	2	3		2	3			3		3		4	4	6	
Penicillium oxalicum Currie and Thom	1	3										3			
Phoma trifolii Johnson et Vall								1				2	2	2	
Rhizopus arrhizus Fischer											1				
Rhizopus nigricans Ehrenberg	1	1	1	2								2	4	4	
Sclerotinia sclerotiorum (Lib.) de Bary		2					1								
Sordaria fimicola (Roberge) Ces. et de Not.	1	3	3	3	3		3						3	2	
Stemphylium botryosum Wallr.	1	1		1			1								
Syncephalastrum racemosus Cohn ex Schröter													1		
Ulocladium consortiale (Thüm.) Simmons												6	7		
Non-sporulating colonies		5	2	4		1	7	2	2	1	3	8	2		
Total	**107**	**108**	**48**	**31**	**14**	**100**	**92**	**48**	**17**	**13**	**101**	**84**	**55**	**48**	**24**
Bacteria					**12**					**15**					**42**

Table 2
Fungi isolated from endophytic tissues of serradella seeds (harvested in 1973) during a 20 year storage

Fungi	Seed batches (numbers)														
	753					754					870				
	Years of storage														
	0	5	10	15	20	0	5	10	15	20	0	5	10	15	20
Absidia corymbifera (Cohn) Sacc.	2				1							1			
Acremoniela atra (Corda) Sacc.						1									
Alternaria alternata (Fr.) Keissler	32	1		1	1	41	8		4	1	49	9	4		1
Aspergillus repens de Bary	1											1		4	
Aureobasidium pullulans (de Bary) Arnaud						1		5	1		2	4			
Chaetomium funicolum Cooke							1						2	2	
Chaetomium globosum Kunze						1									
Cladosporium cladosporioides (Fres.) de Vries							3		3	3	1				
Fusarium oxsysporum (Schlecht.)						2					6				
Fusarium solani (Mart.) Sacc.	1														
Papularia rosea Graben et Kuznetz								3							
Penicillium corymbiferum Westling	4	2	4			2									
Penicillium cyclopium Westling	3					2	1					1			
Phoma trifolii Johnson et Vall		2	2				1						2		
Sclerotinia sclerotiorum (Lib.) de Bary		1					2								
Sordaria fimicola (Roberge) Ces. et de Not.			3					3	1				1		
Non-sporulating colonies		2	3	1	1	1		1		1	3	3			4
Total	**43**	**8**	**12**	**3**	**2**	**50**	**16**	**12**	**9**	**5**	**62**	**18**	**9**	**6**	**2**

CONCLUSIONS

1. The essential agents causing significant reduction of seed-borne fungi of serradella were the storage conditions (low seed moisture – 5.5%, no oxygen) and the long storage term.
2. In the seed-borne fungi communities of serradella two groups were identified:
   a. the short-lived group (up to 5 years): fungi from the genera of *Fusarium* and *Papularia rosea*, *Sclerotinia sclerotiorum*, *Arthrinium arundinis*,
   b. the long-lived group (over 10 years): *Alternaria alternata*, *Absidia corymbifera*, *Acremoniella atra*, *Aspergillus chevalieri*, *A. repens*, *Aureobasidium pullulans*, *Chaetomium funicola*, *Cladosporium cladosporioides*, *Mucor racemosus*, *Penicillium cyclopium*, *P. notatum*, *Phoma trifolii*, *Rhizopus nigricans*, *Sordaria fimicola*, *Stemphylium botryosum*.
3. The degradation of the germinability of the serradella seeds was probably caused by long storage term and infestation by fungi from the genera of *Aspergillus* and *Penicillium*.

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Grzyby występujące na nasionach seradeli podczas 20-letniego przechowywania

Streszczenie

Badano zmiany w zbiorowisku grzybów występujących na nasionach seradeli w czasie długoletniego ich przechowywania. Czynnikami powodującymi znaczną redukcję grzybów występujących na nasionach były: warunki i czas przechowywania (bardzo niska wilgotność nasion, około 5.5%, brak dostępu powietrza).