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Variability of dry seed mycobiota of *Pisum sativum*

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Mycobiota of dry pea seeds of cv. Ramir and line R 429/87 harvested at Radzików, Oleśnica Mała and Łagiewniki in 1991–93 was investigated. Among twenty species *Alternaria alternata* and *Stemphylium botryosum* occurred commonly each year. *Mycosphaerella pinodes*, *Ascochyta pisi*, *Fusarium poae* and *Cladosporium herbarum* were noted on most of the tested seed samples. Percentage of seeds transmitting the fungi varied depending on locations, time and genotypes, being the lowest for Łagiewniki and in 1992. The year 1991 was the most favourable for seed mycobiota development, especially for pathogenic fungi.

Key words: pea seeds, variability of mycobiota.

INTRODUCTION

Fungi transmission by pea (*Pisum sativum* L.) seeds was reported from Canada (S k o l k o et al. 1954), France (A n s e l m e and C h a m p i o n 1962), U. K. (M a t t h e w s 1964) and Poland (G r z e l a k and I ł ł a k o w i c z 1973; C z y ż e w s k a 1976; F i l i p o w i c z 1976). Studies of mycobiota in Poland concerned seeds of green pea (C z y ż e w s k a 1976) as well as field pea (F i l i p o w i c z 1976). The increasing importance of dry edible peas seed production in Poland resulted to the present studies on occurrence and variability of fungi species on this kind of *P. sativum* seeds.

MATERIALS AND METHODS

Seeds of 2 genotypes: cultivar (cv.) Ramir and line R 429/87 were obtained from Plant Breeding and Acclimatization Institute (IHAR) at Radzików

(Central East Poland = CEP), the Experimental Station (ES) of IHAR at Oleśnica Mała (South-West Poland = SWP) and Breeding Station (BS) at Łagiewniki (Central North-West Poland = CNWP). Studies were conducted from 1991 to 1993 with the exception of Oleśnica Mała only in 1992 (Tabs 1–3). Surface sterilized seeds, following K e r r (1963) procedure, were placed on Coon's agar (CN) medium (A l i et al. 1978) into a Petri plate (Pp) 10 cm in diameter. Each Pp contained 12 seeds. The first set of seeds tested in November comprised 204 seeds (17 Pps × 12 sds). The second set of the same number of seeds was evaluated in February. After 3 days under natural day light seeds in Pps were exposed to bulbs with fluorescent day light for the next 4 days for 14 hrs a day. Readings were taken following such incubation. Fungi were identified according to keys (E l l i s 1971; A r x 1974; B o o t h 1977), CMI Descriptions and the work of N o o r d e l o s et al. (1993).

Statistical calculations were done with Statgraphics Plus programme. Incidence of fungi on seeds, after percentage data transformation, was subjected to analysis of variance using F test and means were separated with Tukey multiple range test.

RESULTS

The highest number of fungi species (20) were isolated in 1991 whereas the lowest number of species (12) was noted in 1992 (Tabs 1–3). The quantitative analysis also showed the highest percentage of total fungi in 1991 (161.3%) and the lowest in 1992 (70.9%). When the precentage of fungi was analysed over years, locations and genotypes differences were only statistically documented for locations being the lowest for all the transmitted fungi on seeds from Łagiewniki and the highest from Oleśnica Mała (Tab. 4). The incidence of fungi species varied depending on the number of seed samples regarded to genotype, location or year. Some species occurred commonly and the others incidentally (Tabs 1–3). Variability of fungi occurrence was also noted for percentage of *Alternaria alternata* and *Stemphylium botryosum* commonly occurred during the whole study, while *Mycosphaerella pinodes* only in 1991, *Penicillium* spp. in 1992 and *Fusarium poae* in 1993. *Ascochyta pisi* was observed on seeds quite commonly in 1991 and 1992, *Mycosphaerella pinodes* in 1993 and *Cladosporium herbarum* during the 3 years. In many cases the common occurrence of species was coupled with the higher percentage of seed transmission. This was evident for *M. pinodes* (37.1%) and *A. pisi* (23.6%) in 1991 but the percentage of the latter species was quite high (20.3%) in 1993, even *A. pisi* occurred in 3 out of 6 samples. The other species commonly noted dominated in some the mycobiota of tested seeds, e.g. *A. alternata* from 20.1% in 1992 to 29.3% in 1991 (Tabs 1–3).

Table 1
Fungi on pea seeds harvested in 1991 at Łagiewniki, Oleśnica Mała and Radzików

Species	Percent of seeds transmitting the fungi							Number of samples
	Łagiewniki		Oleśnica Mała		Radzików		Total	
	Ramir	R 429/87	Ramir	R 429/87	Ramir	R 429/87		
Ascochyta pisi Lib.	0.5	2.0	3.9	15.7	–	1.5	23.6	5
Mycosphaerella pinodes (Berk. et Blox.) Vester.	0.5	2.0	5.9	26.2	2.0	0.5	37.1	6
Phoma pinodella (L. K. Jones) Morgan–Jones et Burch	–	1.5	–	–	0.5	0.5	2.5	3
Acremoniella atra Sacc.	–	–	1.0	–	1.0	1.0	3.0	3
Alternaria alternata (Fr.) Keissler	0.7	1.2	9.3	6.1	2.7	9.3	29.3	6
Aspergillus spp.	–	0.5	–	–	1.5	–	2.0	2
Botrytis cinerea Pers.: Fr.	–	–	0.5	–	0.2	0.2	0.9	3
Chaetomium globosum Kunze: Fr.	–	–	–	–	0.5	1.0	1.5	2
Cladosporium herbarum Link: Fr.	–	0.2	–	0.5	0.7	0.7	2.1	4
Epicoccum purpurascens Link	–	–	–	–	–	0.2	0.2	1
Fusarium avenaceum (Corda: Fr.) Sacc.	–	–	2.7	1.7	0.2	–	4.6	3
Fusarium poae (Peck) Woll.	–	–	1.0	–	0.5	1.7	3.2	3
Fusarium spp.	–	1.7	–	0.7	0.5	0.5	3.4	4
Mucor hiemalis Wehm.	–	–	0.2	–	0.5	–	0.7	2
Penicillium spp.	1.2	2.0	5.1	1.5	1.5	–	11.3	5
Sclerotinia sclerotiorum (Lib.) de Bary	–	1.7	–	0.5	–	1.0	3.2	3
Stemphylium botryosum Wallr.	0.2	–	6.1	6.6	4.9	8.1	25.9	5
Trichoderma viride Pers.: Fr.	–	–	0.5	–	4.4	–	4.9	2
Ulocladium sp.	–	–	–	0.5	0.5	0.2	1.2	3
Non-sporulating	–	–	–	–	0.5	0.2	0.7	2
Total	3.1	12.8	36.2	60.0	22.6	26.6	161.3	6
No. of species	5	9	11	10	17	15	20	

Table 2
Fungi occurring on pea seeds collected in 1992 from Łagiewniki and Radzików

Species	Percent of seeds transmitting the fungi				Total	Number of samples
	Łagiewniki		Radzików			
	Ramir	R 429/87	Ramir	R 429/87		
Ascochyta pisi Lib.	–	0.5	0.5	0.5	1.5	3
Mycosphaerella pinodes (Berk. et Blox.) Vester.	–	–	–	1.0	1.0	1
Phoma pinodella (L. K. Jones) Morgan-Jones et Burch	–	–	–	0.5	0.5	1
Alternaria alternata (Fr.) Keis.	1.0	2.5	8.8	7.8	20.1	4
Aspergillus niger Tieg.	1.5	–	–	–	1.5	1
Botrytis cinerea Pers.: Fr.	–	–	1.0	–	1.0	1
Cladosporium herbarum Link: Fr.	0.5	–	3.4	6.9	10.8	3
Fusarium poae (Peck) Woll.	–	0.5	–	1.0	1.5	2
Fusarium sp.	–	0.5	–	0.5	1.0	2
Penicillium sp.	3.4	1.0	1.0	8.3	13.7	4
Stemphylium botryosum Wallr.	4.4	2.5	2.5	4.9	14.3	4
Non-sporulating	1.0	–	0.5	2.5	4.0	3
Total	11.8	7.5	17.7	33.9	70.9	4
No. of species	6	6	7	10	12	

The *Fusarium* species inhabited few seeds; only *F. poae* was encountered each year. The remaining fungi occurred rather incidentally on some seed samples and usually in law amount.

When the incidence of pea pathogenic fungi and commonly occurring saprophytes was analysed over the years, locations and genotypes only in some cases the variability was statistically significant (Tab. 4). The occurrence of *Ascochyta* blight fungi, namely *A. pisi*, *Mycosphaerella pinodes* and *Phoma pinodella*, differed statistically only for locations. The lowest occurrence of these fungi was for seeds harvested at Łagiewniki and Radzików. When the species were considered separately, no significant differences were found for *Mycosphaerella pinodes* and *Phoma pinodella*. In the case of *A. pisi* differences were not only marked between locations, being the lowest for Radzików and the highest for Oleśnica Mała but also between genotypes. Line R 429/87 transmitted more often *A. pisi* than cv. Ramir. Significantly less seeds from Łagiewniki transmitted *A. alternata* to seeds from Radzików and Oleśnica Mała. *Botrytis cinerea* inhabited the highest number of seeds harvested at Radzików but the lowest from Łagiewniki. No significant differences were found for seeds transmitting *S. botryosum* and *Fusarium* spp. (Tab. 4).

Table 3
Fungi on pea seeds collected from Łagiewniki, Oleśnica Mała and Radzików in 1993

Species	Percent of seeds transmitting the fungi							Number of samples
	Łagiewniki		Oleśnica Mała		Radzików		Total	
	Ramir	R 429/87	Ramir	R 429/87	Ramir	R 429/87		
Ascochyta pisi Lib.	–	–	6.4	13.7	–	0.2	20.3	3
Mycosphaerella pinodes (Berk. et Blox.) Vester.	–	0.5	0.5	0.7	–	0.7	2.4	4
Phoma pinodella (L. K. Jones) Morgan–Jones et Burch	0.5	0.5	–	–	–	–	1.0	2
Acremoniella altermatum Link: S. F. Grey	–	–	–	–	–	0.2	0.2	1
Alternaria alternata (Fr.) Keissler	4.2	2.0	6.4	2.9	3.9	6.1	25.5	6
Botrytis cinerea Pers.: Fr.	–	–	0.2	–	0.7	0.5	1.4	3
Chaetomium sp.	–	–	0.2	0.5	–		0.7	2
Cladosporium herbarum Link: Fr.	0.5	–	0.2	0.7	–	0.5	1.9	4
Fusarium poae (Peck) Woll.	2.7	0.7	2.2	2.7	1.2	0.7	10.2	6
Fusarium spp.	–	–	–	0.5	–	0.2	0.7	2
Mucor hiemalis Wehm.	–	–	0.2	2.0	–	–	2.2	2
Penicillium expansum Link: Fr.	–	–	2.9	1.2	–	–	4.1	2
Rhizoctonia solani Kühn	0.5	–	–	–	–	–	0.5	1
Sclerotinia sclerotiorum (Lib.) de Bary	0.7	0.5	–	–	–	1.7	2.9	3
Stemphylium botryosum Wallr.	3.4	2.0	4.9	2.7	0.7	1.5	15.2	6
Non-sporulating	1.0	0.2	1.0	2.7	–	–	4.9	4
Total	13.5	6.4	25.1	30.3	6.5	10.3	92.1	6
No. of species	8	7	11	11	4	10	16	

Table 4

Analysis of variance and multiple range analysis of fungi occurrence on 2 pea genotypes at Radzików (R), Łagiewniki (Ł) and Oleśnica Mała (OM) in 1991–1993

Fungi	Source of variation	D. f.[a]	Mean square	Significance level	Homo[b]	Mean[c]
Total of fungi	Locations	2	0.1026	0.0052	2	0.09 for Ł – 0.40 for OM
Ascochyta pisi *Mycosphaerella pinodes* *Phoma pinodella*	Locations	2	0.0365	0.0106	2	0.01 for Ł (R) – 0.18 for OM
Ascochyta pisi	Locations	2	0.0118	0.0000	2	0.005 for R – 0.099 for OM
	Genotypes	1	0.0045	0.0001	2	0.018 for cv. – 0.054 for line
Alternaria alternata	Locations	2	0.0039	0.0183	2	0.019 for Ł – 0.066 for OM
Botrytis cinerea	Locations	2	3.0412	0.0529	2	0.00 for Ł – 0.004 for R

[a] – Degree of freedom;
[b] – Number of homogeneous groups;
[c] – Means presented from the lowest to the highest one

DISCUSSION

Variability of mycobiota of pea seeds depended on all investigated factors although statistical differences were only found for locations. The localities, seeds were produced, were situated at main edible pea growing area, far away from each other (CNWP, CEP, SWP). That means, different weather conditions may be expected in these locations. The macroclimate of a region of Poland as well as the microclimate of a pea field is affected by existing weather conditions. More humid weather of 1991 caused reacher mycobiota development on seeds than on dry growing season of 1992 (Fig. 1). The occurrence of some fungi species seems to be more dependent on years or localities than other species. The fungi, especially saprophytic one, commonly occurring in the atmosphere are everywhere, so even extreme weather conditions cause not much harm to their existance. The incidence of plant pathogenic fungi looks like to be influenced by the weather conditions existing in a given year or location as majority of them need a drop of free water for their spores to germinate and infect. In consequence, more likely for specific pathogens, as *Ascochyta* complex fungi, their incidence greatly depends on weather conditions for plant infection. These observations were supported by the results of B a t h g a t e et al. (1989) who found seed samples from dry locations of Western Australia to be free of *M. pinodes* and *P. pinodella*. Not only the locations and years but also the genotype may be a factor of mycobiota variability. In these studies only the incidence of *A. pisi* differed considerably between tested cv. and line.

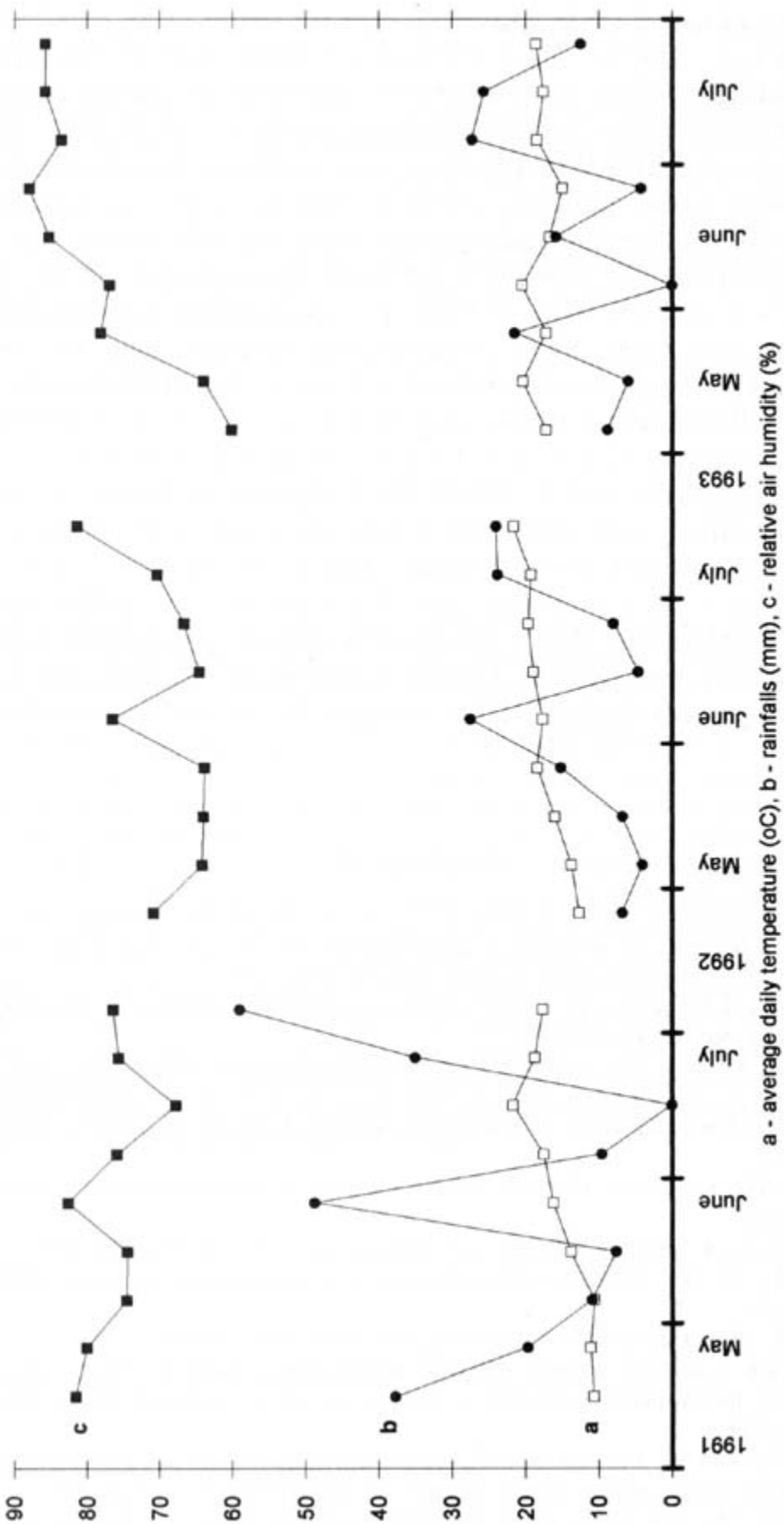

Fig. 1. Weather characteristics during the growing season of 1991–1993 at Radzików

The data obtained are in agreement with the results of Grzelak and Iłłakowicz (1973), Czyżewska (1976) and Filipowicz (1976) indicating common occurrence of *A. alternata* on different cultivars of pea. *S. botryosum* was also isolated frequently from seeds by Grzelak and Iłłakowicz (1973). These findings were confirmed by presented results. All the authors found *Ascochyta* complex fungi on seeds but quantitatively their results differed greatly. Studies conducted in the 1970 showed that *A. pisi* dominated on seeds, but recently, it has been demonstrated that *M. pinodes* prevails (Marcinkowska 1996). The opinions also varied on *Fusarium* incidence on pea seeds. These investigations revealed only the presence of 2–3 species on pea seeds while Czyżewska (1976) and Filipowicz (1976) found 7 species and Marcinkowska (1993) noted 5 species of *Fusarium*. Czyżewska (1976) and Filipowicz (1976) isolated *F. oxysporum* and *F. solani*, the dangerous pathogens of pea, but I obtained mainly *F. poae* which was frequently noted by the above authors. This species dominated also in earlier studies of Marcinkowska (1993). Czyżewska (1976) and Filipowicz (1976) described up to 50% more species than I did but the number of genera to which the species belonged was similar. Species composition of fungi infested pea seeds was in general similar to that obtained by Skolko et al. in Canada (1954).

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Zmienność mikoflory nasion *Pisum sativum*

S t r e s z c z e n i e

Nasiona grochu jadalnego, uprawianego na suche nasiona, wykładano do szalek Petriego (ϕ 10 cm) na pożywkę mineralną Coon'a. Nasiona odmiany Ramir i linii hodowlanej R 429/87 zebrane w Radzikowie (Mazowsze) i Łagiewnikach (Kujawy) w latach 1991–93 oraz w Oleśnicy Małej (Dolny Śląsk) z 1991 i 1993 stanowiły przedmiot badań. Próba każdej kombinacji obejmowała 408 nasion, badanych w dwóch seriach, każda 204 nasiona (17 sz. × 12 n), odległych w czasie o 2 miesiące. Procent nasion zasiedlonych przez grzyby różnił się zależnie od miejsca, lat i genotypów. Statystycznie udowodnione różnice stwierdzono tylko dla pierwszego czynnika z najniższym ogólnym procentem grzybów przenoszonych na nasionach z Łagiewnik i najwyższym z Oleśnicy Małej. Najmniej grzybów (liczba gatunków i procent zasiedlenia) znaleziono na nasionach z roku 1992 a najwięcej z 1991. Spośród stwierdzonych tam 20 gatunków, *Alternaria alternata* i *Stemphylium botryosum* występowały powszechnie, co roku, zaś *Mycosphaerella pinodes*, *Ascochyta pisi*, *Fusarium poae*, w niektórych latach. Ostatnie 3 gatunki a także *Cladosporium herbarum* i *Penicillium* spp., zasiedlały większość badanych próbek nasion. Pozostałe gatunki występowały w niektórych próbach, osiągając maksymalną wartość 4.4% (*Trichoderma viridae*); 2.9% (*Penicillium expansum*), 2.7% (*Fusarium avenaceum*) i 1.7% (*Sclerotinia sclerotiorum*) zasiedlenia nasion. Wskazuje to na zjawisko przypadkowości w występowaniu różnych grzybów na nasionach. Wydaje się też, że występowanie grzybów niepatogenicznych jest mniej uzależnione od rejonu uprawy roślin aniżeli patogenów roślin.