ACTA MYCOLOGICA
Vol. 32 (1): 31–39
1997

Micromycetes on *Pisum sativum* var. *arvense*

JOANNA MARCINKOWSKA

Department of Plant Pathology, Warsaw Agriculture University
02-787 Warsaw, Nowoursynowska 166, Poland

M a r c i n k o w s k a J.: Micromycetes on *Pisum sativum* var. *arvense*. Acta Mycol. 32 (1): 31–39, 1997.

Seeds of 11 Austrian winter pea genotypes, harvested at Radzików (CEP) in 1993 and 1994, were evaluated for fungi occurrence on Coon's agar medium in Petri plates. Number of species isolated depended on the genotype and year of collection. *Alternaria alternata*, *Stemphylium botryosum* were found on all the tested samples and *Phoma pinodella* and *Fusarium poae* were also common while *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Mycosphaerella pinodes* appeared to be common only in 1993. Three species occurred only once. The mycoflora was richer in 1993. The common seed inhabitants usually transmitted higher percentage of fungi than species occuring more seldom.

Key words: micromycetes on *Pisum sativum*.

INTRODUCTION

Several studies were conducted with *Pisum sativum* L. seed mycoflora of spring-planted peas (M a r c i n k o w s k a 1998). Some authors (S k o - l k o et al. 1954; T r u s z k o w s k a et al. 1968; C z y ż e w s k a 1976; F i l i p o w i c z 1976) investigated not only pathogenic fungi but also saprotrophic ones as the latter might cause seed destruction, thus decreasing plant stands (F i l i p o w i c z 1976; M a r c i n k o w s k a, S c h o l l e n - b e r g e r 1979). Recently Polish plant breeders at Radzików (personal communication) have bred a few lines of fall-planted Austrian winter pea, *Pisum sativum* L. var. *arvense* (L.) Poir.

The objective of this study was to describe all the fungi transmitted by seeds of the newly introduced pea crop into Poland in order to become acquainted with the total spectrum of fungi occurring in the above environment.

MATERIAL AND METHODS

Seeds were harvested at Radzików (Central-East Poland = CEP) in the Plant Breeding and Acclimatization Institute at Radzików from Austrian winter pea plots. In 1993 one cultivar and 7 breeding lines (Table 1) were evaluated while in 1994 the same cv. Melrose and 2 breeding lines R 1 and R 2 were tested along with 3 new lines (Table 2). Agar plate method with Coon's (CN) medium (Ali et al. 1978) in Petri plate (Pp) 10 cm diameter was used. Twelve surface sterilized seeds, following K e r r's (1963) procedure, were placed in a Pp. A set of a sample contained 204 seeds (17 Pp × 12 sds). A whole sample comprised 408 seeds. Seed samples were divided to be evaluated at different time, the first set in the late fall and the second one in the middle of winter. The same incubation conditions were provided for seeds of both sets (M a r c i n k o w s k a 1997). Readings were taken on the eighth day since planting.

The keys of E l l i s (1971); Arx (1974), B o o t h (1977) and CMI Descriptions and the paper (N o o r d e l o o s et al. 1993) were used for fungi identification.

Data were statistically evaluated with Statgraphics Plus programme. Fungi frequency on seeds after percentage data transformation was evaluated by analysis of variance using F test and means were separated with Tukey multiple range test.

RESULTS

Austrian winter pea seeds harvested in 1993 transmitted higher number of fungi species than those of 1994, respectively 20 and 16. The last year 6 genotypes were evaluated while in 1993 — 8 ones (Tables 1-2). The three genotypes studies both years showed more numerous fungal species occurrence in 1993. Apart from the number of species the percentage of seeds occupied by the fungi was also higher in 1993 than in 1994, as well as for cv. Melrose and lines R 1, R 2, but these differences were statistically non-significant (Table 3). *Alternaria alternata* and *Stemphylium botryosum* were the only species which occurred on all the seed samples tested in 1993 and 1994 (Tables 1-2). *Phoma pinodella* and *Fusarium poae* (absent on one and two out of 14 samples, respectively) were the next common inhabitants of the seeds. *Sclerotinia sclerotiorum* and *Botrytis cinerea* occurred on majority of samples (7/8) in 1993 but on half of them (3/6, 2/6) in 1994. *Ascochyta pisi* and *Mycosphaerella pinodes* were also noted less frequently in 1994. The other 12 species were isolated from less than a half of the samples but 3 of them (*F. oxysporum*, *Trichothecium roseum*, *Ulocladium consortiale*) occurred only once.

Table 1
Occurrence of fungi on seeds of 8 pea genotypes harvested at Radzików in 1993

Species	Percetage of seeds (%) transmitting a species								Total	Number of samples
	Melrose	R 1	R 2	59/2	L 61/3	L 61/4	L 63/4	L 63/6		
Ascochyta pisi Lib.	–	–	0.25	0.25	–	1.72	–	0.49	2.71	4
Mycosphaerella pinodes (Berk. et Blox.) Vester.	–	–	0.49	7.84	2.21	9.80	–	9.07	29.41	5
Phoma pinodella (L.K. Jones) Morgan-Jones et Burch	0.74	0.25	–	0.98	0.74	0.74	0.49	1.96	5.9	7
Alternaria alternata (Fr.) Kreis.	5.39	1.47	2.94	3.68	1.47	0.49	3.43	7.11	25.98	8
Botrytis cinerea Pers:. Fr.	0.49	0.74	–	0.49	0.98	0.74	0.49	0.49	4.42	7
Chaetomium globosum Kunze: Fr.	–	0.25	–	–	–	–	–	–	0.25	1
Cladosporium herbarum Link: Fr.	–	0.25	–	0.25	–	0.49	–	–	0.99	3
Fusarium avenaceum (Corda: Fr.) Sacc.	–	–	–	0.98	0.49	0.25	–	–	1.72	3
Fusarium culmorum (W.G. Smith) Sacc.	–	–	0.25	–	–	0.25	–	–	0.5	2
Fusarium oxysporum Schl.	–	–	–	–	–	–	0.49	–	0.49	1
Fusarium poae (Peck) Woll.	1.71	0.98	0.74	16.67	4.41	4.17	0.74	2.21	31.63	8
Fusarium solani (Mart.) Sacc.	–	–	–	–	0.49	0.74	–	–	1.23	2
Penicillium spp.	0.25	–	0.25	–	0.49	–	–	–	0.99	3
Phoma herbarum West.	–	0.25	0.25	–	–	0.25	–	–	0.75	3
Rhizoctonia solani Kühn	–	–	0.49	–	–	–	–	–	0.49	1
Rhizopus sp.	–	–	0.25	–	–	–	–	–	0.25	1
Sclerotinia sclerotiorum (Lib.) de Bary	1.96	0.49	0.74	0.74	–	0.25	1.47	0.25	5.9	7
Stemphylium botryosum Wallr.	1.96	0.98	1.23	0.74	1.47	0.25	2.21	1.96	10.8	8
Trichothecium roseum Link	–	–	–	0.25	–	–	–	–	0.25	1
Non-sporulating	–	0.25	0.74	–	–	–	0.49	–	1.48	3
Total	12.5	5.91	8.62	32.87	12.75	20.14	9.81	23.54	126.14	
No. of species	7	10	12	11	9	13	7	8	20	

Table 2
Incidence of fungi on seeds of 6 pea genotypes harvested at Radzików in 1994

Species	Percentage of seeds (%) transmitting a species						Total	Number of samples
	Melrose	R 1	R 2	227/87/4	344/87/3	227/87/6		
Ascochyta pisi Lib.	–	–	–	1.96	0.25	–	2.21	2
Mycosphaerella pinodes (Berk. et Blox.) Vester.	–	–	–	6.86	0.49	–	7.35	2
Phoma pinodella (L.K. Jones) Morgan-Jones et Burch	1.23	0.25	0.25	12.01	19.85	0.25	33.84	6
Alternaria alternata (Fr.) Kreis.	2.21	1.72	1.96	3.19	1.72	1.23	12.03	6
Botrytis cinerea Pers: Fr.	–	0.25	–	0.74	–	–	0.99	2
Chaetomium globosum Kunze: Fr.	–	–	1.72	0.74	0.25	0.25	2.96	4
Cladosporium herbarum Link: Fr.	–	–	–	–	–	0.25	0.25	1
Fusarium poae (Peck) Woll.	–	0.25	0.49	0.74	2.45	–	3.93	4
Penicillium sp.	–	0.25	–	–	0.49	0.98	1.72	3
Phoma herbarum West.	–	–	–	0.25	–	–	0.25	1
Rhizoctonia solani Kühn	–	0.25	0.25	1.23	–	–	1.73	3
Rhizopus sp.	0.25	0.25	–	–	–	–	0.5	2
Sclerotinia sclerotiorum (Lib.) de Bary	0.25	0.49	–	0.49	–	–	1.23	3
Stemphylium botryosum Wallr.	0.74	1.72	0.74	0.98	0.49	0.25	4.92	6
Ulocladium consortiale (Thüm.) Sim.	–	–	–	0.25	–	–	0.25	1
Non-sporulating	0.25	–	–	0.25	–	–	0.5	2
Total	4.93	5.43	5.41	29.69	25.99	3.21	74.66	
No. of species	6	9	6	13	8	6	16	

The variation of fungal occurence was also manifested in the percentage of species transmission. In 1993 *F. poae* (31.63), *M. pinodes* (29.41) and *A. alternata* (25.98) inhabited the highest percentage of seeds, while *Chaetomium globosum*, *T. roseum* and *Rhizopus* sp. only 0.25%. *P. pinodella* was the most frequently occurring fungus (33.84) in 1994, being isolated almost 3 times as often as *A. alternata* (12.03) while *Cladosporium herbarum* and *U. consortiale* occurred on only 0.25% of seeds. The differences in fungal transmission were noted as well between genotypes. However, statistical differences were marked in a few cases, such as total of *A. pisi, M. pinodes and P. pinodella*, the species responsible for ascochyta blight disease' and separately *P. pinodella* (Table 3). In both cases line R 1 was the slightest inhabited but the lines 227/87/4 and 344/87/3 were the most infected. When the total percentage of pea pathogens (*A. pisi*, *M. pinodes*, *P. pinodella*, *B. cinerea*, *F. oxysporum*, *F. solani.*, *Rhizoctonia solani*, *S. sclerotiorum*) was considered statistical differences were found, line 227/87/6 being inhabited the least and the highest one 227/87/4 (Table 3). Statistically significant differences were also found for the species of *Fusarium* and *F. poae* most commonly occurring among the genera (Table 3). Line 227/87/6 was not inhabited by *Fusarium* spp. but line 59/2 transmitted the highest percentage of *F. poae* whereas line 63/4 the lowest percentage. No statistical differences existed between genotypes in their ability for *A. alternaria*, *B. cinerea* and *S. sclerotiorum* transmission.

DISCUSSION

Austrian winter pea seed mycoflora was investigated in the same way as it was done for dry pea seeds (Marcinkowska 1998). The agar plate method recommended by several laboratories (Hewett 1987) was effective here. CN agar was used instead of PDA (Grzelak, Iłłakowicz 1973) or MA (Hewett 1987) because of very good fungi sporulation and clear readings on the first medium (Marcinkowska 1997).

Composition of species occurring on spring pea seeds (Truszkowska et al. 1968; Marcinkowska 1998) and Austrian winter pea seeds was similar, especially for plant pathogenic fungi (Marcinkowska 1998). However, in the present study two more pea pathogens, i.e. *F. oxysporum* and *F. solani*, were isolated from seeds. These species had already been noted on edible pea seeds (Czyżewska 1976; Filipowicz 1976; Marcinkowska 1993).

The fungi commonly occurring on spring pea seeds (Marcinkowska 1998) were also present on the majority of the tested samples of fall-planted peas. The percentage of the common seed inhabitant differed for separate studies since genotypes, locations and years varied for them (Czyżewska 1976; Filipowicz 1976; Marcinkowska 1998).

Table 3

Analysis of variance and multiple range analysis of fungi occurrence on 11 pea genotypes at Radzików in 1993 and 1994

Fungi	Source of variation	Degree of freedom	Mean square	Significance level	No of homogenous groups	Mean – from the lowest to highest one
Ascochyta pisi, Mycosphaerella pinodes, Phoma pinodella	Genotypes	10	74.0423	0.0016	3	0.25 for R 1 – 20.3 for 227/87/4
Phoma pinodella	Genotypes	10	35.1728	0.0009	3	0.12 for R 1 – 19.73 for 344/87/3
Total of pathogens	Genotypes	10	77.3380	0.0040	5	0.82 for 227/87/6 – 23.86 for 227/87/4
Total of *Fusarium* spp.	Genotypes	10	22.6788	0.0092	2	0.49 for 227/87/6 – 17.16 for 59/2
Fusarium poae	Genotypes	10	20.3220	0.0137	2	0.29 for L 63/4 – 16.28 for 59/2

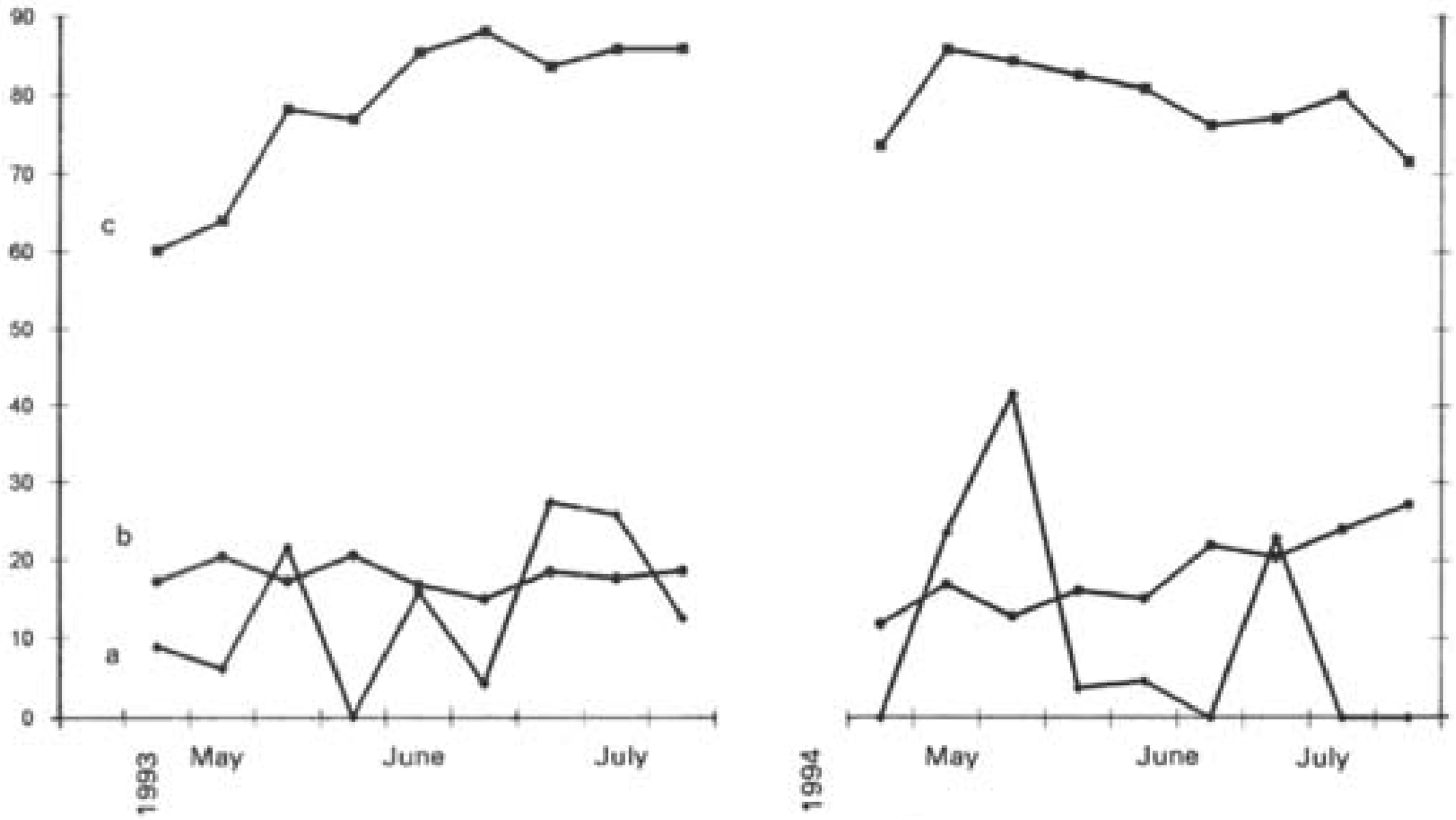

Fig. 1. Weather characteristics during 1993 and 1994 growing season (May, June, July) at Radzików
a — total rainfall (mm), *b* — average daily air temperature (°C), *c* — average humidity (%)

Some investigations (Czyżewska 1976; Marcinkowska 1998), revealed that *A. alternata* dominated on seeds while others (Grzelak, Iłłakowicz 1973; Filipowicz 1976) -that. *A. pisi*. More recently *M. pinodes* and *P. pinodella* (Bathgate et al. 1989; Marcinkowska, Szyrmer 1992; Marcinkowska 1998) were found very often. In these studies *P. pinodella* was isolated from the highest percentage of seeds in 1994 while in 1993 *M. pinodes*, and *F. poae*. These differences resulted from genotype and enviroment variability as some genotype seeds were more frequently infested by the fungi in comparison to others. The weather of spring and early summer in 1993 and 1994 was quite similar but with heavier rainfalls in May 1994 and slightly higher RH in July 1993 (Fig. 1). These factors might have influenced the micromycetes but they were not statistically significant. In general, Austrian pea seed mycobiota was variable like on spring-planted peas, with the tendency of more common occurrence of *P. pinodella* reather than *A. pisi* or *M. pinodes*, the fungi responsible for ascochyta blight disease'.

REFERENCES

Ali S. M., Nitsche L.F., Dube A.J., Krause M. R., Cameron B. 1978. Selection of pea lines for resistance to pathotypes of *Ascochyta pinodes*, *A. pisi* and *Phoma medicaginis* var. *pinodella*. Aust. J. Agric. Res. 29: 841-849.

Arx von J. A. 1974. The genera of fungi sporulating in pure culture. J. Cramer, Vaduz.

Bathgate A., Sivasithamparam K., Khan T. N. 1989. Identity and recovery of seed-borne fungal pathogens of field peas in Western Australia. N. Zeal. J. Crop. Hort. Sci. 17: 97-101.

Booth C. 1977. Laboratory guide to the identification of the major *Fusarium* species. C.M.I., Kew. Surrey.

CMI Descriptions of Pathogenic Fungi and Bacteria No. 150, 334, 340, 406, 431, 513.

Czyżewska S. 1976. Studies on fungi infesting seeds of pea (*Pisum sativum* L.). Biul. Warz. 19: 271-288.

Ellis M. B. 1971. Dematiaceous *Hyphomycetes*. CMI, Kew, Surrey.

Filipowicz A. J. 1976. Badania mikoflory nasion grochu siewnego (*Pisum sativum* L.) ze szczególnym uwzględnieniem grzybów z rodzaju *Ascochyta* i *Fusarium*. Roczn. Nauk Roln. ser. E 5 (2): 85-119.

Grzelak K., Iłłakowicz A. 1973. Grzyby z rodzaju *Ascochyta* w laboratoryjnej ocenie zdrowotności nasion grochu (*Pisum sativum* L.). Biul. IHAR 3-4: 114-115.

Kerr A. 1963. The root rot *Fusarium* wilt complex of peas. Austral. J. Biol. Sci. 16: 55-59.

Marcinkowska J. Z. 1993. *Fusarium* spp. as pathogens of dry peas (*Pisum sativum* L.) in Poland. Hod. Rośl. Aklim. Nasien. 37 (4): 95-102.

Marcinkowska J. 1998. Variability of dry seed mycobiota of *Pisum sativum*. Acta Mycol. (in print).

Marcinkowska J. 1997. Diagnostyka sprawców zgorzelowej plamistości grochu. Biul. IHAR (in print).

Marcinkowska J., Schollenberger M. 1979. Mikroflora nasion soi. Roczn. Nauk. Roln. ser. E, 9 (2): 41-54.

Marcinkowska J., Szyrmer J. 1992. Etiologia zgorzeli siewek grochu ozimego (*Pisum sativum* L. var. *arvense* (L.) Poir.). Biul. IHAR 184: 89-92.

Noordeloos M. E., De Gruyter J., Van Eijk G. W., Roeijmans H. J. 1993. Production of dendritic crystals in pure cultures of *Phoma* and *Acsochyta* and its value as a taxonomic character relative to morphology, pathology and cultural characteristics. Mycol. Res. 97 (11): 1343-1350.

Skolko A. J., Groves J. W., Wellen V. R. 1954. *Ascochyta* diseases of peas in Canada with special reference to seed transmision. Can. J. Agric. Sci. 34 (4): 417-428.

Truszkowska W., Jasa S., Józefowicz M., Ušak P. 1968. Obserwacje mikroflory nasion niektórych roślin przechowywanych bez dostępu powietrza. Biul. IHAR 1968 (1-2): 165-182.

Micromycetes nasion grochu zimującego

Streszczenie

W 1993 roku badano występowanie grzybów na nasionach 8 genotypów grochu zimującego (*Pisum sativum* L. var. *arvense* (L.) Poir.), zaś w 1994 na nasionach 6 genotypów uprawianych w Radzikowie. W obydwu latach oceniano tylko nasiona odmiany Melrose oraz linii R 1 i R 2. Wyniki testu szalkowego na pożywce Coon'a wskazały na występowanie *Alternaria alternata*

i *Stemphylium botryosum* we wszystkich próbach nasion. *Phoma pinodella* i *Fusarium poae* izolowane były równie powszechnie tak jak: *Botrytis cinerea*, *Sclerotinia sclerotiorum* i *Mycosphaerella pinodes*, ale 3 ostatnie gatunki tylko w 1993 roku. Z kolei *F. oxysporum*, *Trichothecium roseum* i *Ulocladium consortiale* stwierdzono tylko na nasionach z pojedynczych prób. Liczba gatunków grzybów a także procent zasiedlonych przez nie nasion różnił się w zależności od próby (rok, genotyp). Statystycznie udowodnione różnice stwierdzono pomiędzy genotypami dla sprawców askochytozy grochu (*Ascochyta pisi*, *M. pinodes P. pinodella*), gatunku *P. pinodella*, grzybów patogenicznych (*A. pisi*, *M. pinodes*, *P. pinodella*, *B. cinerea*, *F. oxysporum*, *F. solani*, *Rhizoctonia solani*, *S. sclerotiorum*) oraz gatunków rodzaju *Fusarium* i *F. poae*. Mikoflora nasion była bogatsza w 1993 r. Na nasionach z poszczególnych genotypów przenoszone były grzyby z 6-13 gatunków. Najwyższy procent (33,84) nasion zasiedlonych przez *P. pinodella* stwierdzono w 1994 r. Skład gatunkowy mikoflory nasion grochu zimującego był podobny do stwierdzonego na nasionach *P. sativum* z upraw wiosennych, zwłaszcza dla grzybów fitopatogenicznych. Ilościowe ich występowanie zmieniało się w zależności od genotypów i warunków otoczenia (pole doświadczalne, warunki atmosferyczne, rok).