

## Fungi of the genus *Penicillium* on apples and pears during the storage period

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### Abstract

Isolation of *Penicillium* fungi in 1391 cases confirmed their presence on and pathogenicity to apples and pears. Four species were the cause of rotting of the fruits: *P. expansum*, *P. diversum*, *P. cyclopium* and *P. spinulosum*. All those species may occur separately or coexist in the mould spots. *P. expansum* spores may infect fruit with injured skin, only if they have an additional source of sugar and nitrogen.

### INTRODUCTION

Fungi of the genus *Penicillium* are common in nature. Many investigators have isolated them from various organic and inorganic materials. Most numerous, however, is the group living and developing in the soil.

Investigations on fungi of the genus *Penicillium* were performed by Zaleski (1927) who described a number of new species occurring in various soils in Poland. This author reported many detailed and accurate observations concerning the principles of identification of species within the genus *Penicillium* on the basis of the conidiophore structure. The American monograph of the genus *Penicillium* by Thom and Raper (1949) appeared much later. More recent studies describe a most several species and do not cover the whole problem (Smith 1963; Onions 1966).

Fungi of the genus *Penicillium* are known to cause rotting of fruits in storage. *Penicillium expansum* Link is in general considered the most dangerous pathogen of stored fruit, particularly apples and pears. This common view, however, proved erroneous, since other species of the same genus also develop on the mould spots usually caused by fungi of the genus *Penicillium*. It was also found that *Peni-*

*cillium expansum* Link., although common, is not capable under normal conditions of infecting fruit through the intact epidermis (Borecka 1962). The wide range of occurrence for the genus *Penicillium* suggests the probability of existence of several species of these fungi which parasitize stored fruit.

A precise identification of fungi of the genus *Penicillium* became indispensable in connection with the detection of mycotoxins produced in the tissues of rotting fruits owing to their infection by fungi of the genus *Penicillium*. Recent investigations demonstrated that formation of metabolites of the nature of mycotoxins is a characteristic trait of this fungus genus. The fungus common on apples, *P. expansum*, produces patulin mycotoxin with a strong carcinogenic action (Beer 1974; Hesseltine 1973; Summer 1974).

The present studies were undertaken to obtain a closer knowledge of fungi of the genus *Penicillium* occurring on stored fruit, apples and pears, in Poland and to establish the pathogenicity of the particular species and strains.

#### I. OCCURRENCE OF FUNGI OF THE *PENICILLIUM* ON STORED APPLES AND PEARS IN POLAND

##### Material and methods

Investigations on the occurrence of fungi of the genus *Penicillium* were started in 1964. Fungi were isolated from representative samples of rotting apples and pears, consisting of two 20 kg boxes of fruit each and at least 5 chosen varieties. From the fruit sample 20 isolates were taken, each from a different fruit. The apple samples from which the fungi were isolated came from 17 orchards and were stored in 17 storage houses in different parts of Poland. Fungi were isolated from the following apple varieties: Antonówka, Bancroft, Boiken, Cortland, Jonathan, Kronselska, Landsberger Reinette, Linda, Linneus' Pippin and Starking; the following pear varieties were examined: Hardie's Beurré, Bonchretien Williams, Favourite, Doyenne du Comis, Lucas, Minister Lucius and Plebanka.

The apples were taken from the following localities: Baucyny near Olsztyn, Brzezna near Nowy Sącz, Dąbrowice near Skierniewice, Dębno-Wola near Grójec, Doły Michałkowskie near Sandomierz, Domaników near Kutno, Gorzędziej near Tczew, Lubianków near Głowno, Nowa Wieś near Warka, Przybroda near Poznań, Przytoczno near Radzyń, Radzięcin near Biłgoraj, Siejnik near Suwałki, Sinołęka near Siedlce, Skierniewice, Warka, Wolica-Ursynów — Warsaw. The pears from which

*Penicillium* was isolated came from Dąbrowice and Skierniewice and were stored in Skierniewice in cold storage at various temperatures.

From the isolates of each fungus culture one-spore cultures were prepared by the method of gradual dilutions in order to identify the species. From each isolate at least 5 one-spore cultures were prepared to exactly describe the morphological differences between the isolates.

A total of 1095 isolates were taken from apples and 296 from pears of the frequently occurring 4 species of the genus *Penicillium*: *P. expansum*, *P. diversum*, *P. cyclopium* and *P. spinulosum*.

### Results

The number of isolates from apple for the particular species obtained in each locality is shown in Table 1. From all localities most frequent was the species *P. expansum*, next came *P. diversum*, *P. cyclopium* was third, and *P. spinulosum* fourth. Only from two localities was *P. diversum* more frequent than *P. expansum* (Baucyny near Olsztyn and Domaników near Kutno). From the remaining localities *P. expansum* prevailed. A total of 668 *P. expansum* isolates 252 of *P. diversum*, 102 of *P. cyclopium* and 78 of *P. spinulosum* was obtained from apples.

Table 1

Number of isolates of four species of the genus *Penicillium* isolated from apples picked from 17 orchards and stored in 17 storage houses in Poland

Locality	Fungus species			
	<i>P. expansum</i>	<i>P. diversum</i>	<i>P. cyclopium</i>	<i>P. spinulosum</i>
Baucyny	43	55	21	12
Brzezna	31	15	—	6
Dąbrowice	26	16	13	3
Dębno-Wola	20	8	6	2
Domaników	13	20	7	3
Doły Michałkowskie	73	30	6	7
Gorzędziej	12	6	2	1
Lubianków	36	22	9	4
Nowa Wieś	44	10	2	2
Przybroda	34	13	4	2
Przytoczno	3	—	1	—
Radzięcin	24	6	4	—
Siejnik	39	—	2	1
Sinołęka	40	18	11	4
Skierniewice	81	23	7	21
Warka	48	5	3	3
Wolica-Ursynów	41	5	4	2
Total	668	252	102	73

A total of 1095 isolates were collected from apples.

Table 2

Number of isolates of four species of the genus *Penicillium* isolated from pears stored in Skierniewice, picked from the orchards in Dąbrowice and Skierniewice

Locality	Fungus species			
	<i>P. expansum</i>	<i>P. diversum</i>	<i>P. cyclopium</i>	<i>P. spinulosum</i>
Dąbrowice	38	14	3	7
Skierniewice	121	63	37	13
Total	159	77	40	20

A total of 296 isolates were collected from pears.

The results obtained from the fungi isolation on pears are shown in Table 2. Most abundant proved to be, as on apples *P. expansum*. The sequence of species was as follows: 159 isolates of *P. expansum*, 77 of *P. diversum*, 40 of *P. cyclopium* and 20 of *P. spinulosum*.

Since pears are seldom stored, isolations could only be made on fruits from two localities, kept in the cold storage in Skierniewice.

## II. SEARCH FOR THE SOURCES OF INFECTION OF THE FRUIT

Observations were undertaken to find the source of fruit infection. Disinfection of the storage rooms recommended by some and criticized by others made it necessary to search for primary sources of infection.

### Material and methods

Fungi were isolated from the air in the storage, rooms in the cold storage areas, in the orchards, and from the soils in these orchards.

Fungi were isolated from the air in the orchards in Sinołęka, Nowa Wieś and Skierniewice. This was done by means of open Petri dishes with solid sterile agar. At each site 10 dishes were set for 1 h. After several days the developing fungus cultures were separated. Then, by the method of gradual dilutions one-spore cultures were prepared, at least five from each culture.

The search for infection sources in the crown of fruit trees was conducted by the method of placing pieces of bark, small knots or buds on the dishes with nutrient medium.

The soil fungi were isolated from samples washed with steril water. This was done in the orchards of Nowa Wieś, Sinołęka and Skierniewice.

### Results

The results are shown in Table 3. The fungus most frequently isolated from air was *P. expansum* in cold storage, in normal storage and in the orchard, but it was not found in the soil. A total of 229 isolates

of *P. expansum* was collected from the air. Second as regards frequency came *P. diversum*, isolated not only from the air in the orchard and storage area, but also from the soil. A total of 108 isolates of *P. diversum* were taken from the air. *P. cyclopium* was less frequent, particularly in the cold storage with temperature of 0°C and it was not found in the soil. A total of 30 isolates of this fungus were collected. *P. spinulosum* was found in the air, more frequently than *P. cyclopium*, particularly in the cold storage at 0°C. A total of 41 isolates were obtained and the fungus was also found in the orchard soil.

Table 3

Number of isolates of the particular fungus species obtained from the air and soil

Fungus species	Place of isolation					
	cold storage		no mal storage	orchard	total from air	soil
	0°C	0°C				
<i>Penicillium expansum</i>	84	67	61	17	229	—
<i>Penicillium diversum</i>	43	28	31	6	108	3
<i>Penicillium cyclopium</i>	4	11	11	4	30	—
<i>Penicillium spinulosum</i>	18	11	8	4	41	2
Total	149	117	111	31	408	5

Total number of isolates of particular fungus over-all number of isolates 821.

From the air and soil 821 isolates were collected. Isolation from the tree crown did not give positive results. Not one fungus of the genus *Penicillium* was found.

### III. THE PROCESS OF APPLE INFECTION BY THE FUNGUS *P. EXPANSUM*

It was attempted to elucidate why *P. expansum* is unable to infect apples through uninjured epidermic.

#### Material and methods

Infection trials were performed on uninjured apples of Glogierówka and Wealthy cvs., ten fruits of each in every combination. The experiments were replicated three times. The following types of inoculum were tested: aqueous suspension of spores and dry spores of the fungus and mycelium.

Experimental combinations: 1. inoculum: spore suspension in 2 per cent glucose, in water, in apple juice, in filtrate of 7 day culture of the fungus *Gloeosporium perennans* (*Pezicula malicorticis*), in filtrate of an aqueous suspension of spores of the same fungus in which the spores had been kept for 4 h, in filtrate of aqueous suspension of spores.

of the fungus *Botrytis cinerea* after soaking the spores for 12 h, and in liquid Czapek's medium; 2. inoculum: dry fungus spores placed on: a piece of apple infected by the fungus *Gloeosporium perennans* (*Pezizula malicorticis*), on a cube of malt extract agar medium containing 2 percent of dry mass, with raw grated apple, and a cube of Czapek's medium, with a cube of rotting apple infected with *P. expansum*, as well as spores placed directly on an injured and an uninjured apple. The medium cubes and pieces of apple were attached to the apple surface with adhesive tape, grated apple was placed on the test apple and covered with a sterile moist piece of absorbent cotton the whole was attached with adhesive tape; 3. inoculum: mycelium — was only tested on injured and uninjured apples.

Infection with spore suspension was done by means of sterile absorbent cotton swabs dipped in the tested inoculum and attached with adhesive tape to the apple surface.

The fruits were stored in containers with a high humidity at 20°C in order to ensure optimal conditions for infection.

Analogously, the influence of the particular components of Czapek's medium added to the spore suspension was tested. The components were used in an amount corresponding to their concentration in the medium. The influence of each particular component was tested as well as combinations of these components with sucrose and also medium solidified with agar and liquid medium with one component lacking. Fungus growth on Czapek's medium with one component missing was evaluated according to a 5-degree comparative scale: 5 — normal growth, colour and sporulation; 4 — good growth, colour unchanged, formation of numerous coremia; 3 — poor growth, light green colour, culture diffuse, centre normally coloured; 2 — very poor growth, white colour, culture diffuse, centre green; 1 — culture very poor white, diffuse.

### Results

The results obtained in tests of the influence of various types of inoculum on uninjured apples are shown in Table 4. In the case of application of a spore suspension of *P. expansum*, the best results were obtained with liquid Czapek's medium (100% of fruits rotted). Quite good results were obtained when filtrate from the aqueous spore suspension of *Gloeosporium perennans* was added (70% of fruits rotted). Results after application of filtrate from this fungus culture were only half as large (45% fruits rotted). *P. expansum* spore suspension in apple juice caused decay of only 14 per cent of the fruits. When water, glucose and *Botrytis cinerea* spore filtrate were applied the fruits were not infected if the epidermis was uninjured.

When inoculum was used in the form of dry *P. expansum* spores

Table 4

Comparison of various methods of uninjured apple inoculation (Wealthy cv.) with *P. expansum*. Average per cent of fruits rotting after infection

Kind of inoculum	Combinations	Rotting fruits %
Spore suspension	in water	0 a
	in 2% glucose	0 a
	in filtrate of aqueous suspension of <i>Botrytis cinerea</i> spores	0 a
	in apple juice	14.3 ab
	in filtrate of <i>Gloeosporium perennans</i> culture	45.0 b
	in filtrate of aqueous suspension of <i>Gloeosporium perennans</i> spores	70.0 bc
	in liquid Czapek's medium	100.0 c
Spores	on uninjured apples	0 a
	with cube of rotting apple ( <i>P. expansum</i> )	7.5 a
	with cube of malt extract agar medium (2% dry weight)	10.0 a
	with grated apple	17.0 ab
	with cube of rotting apple ( <i>G. perennans</i> )	25. ab
	with cube of Czapek's medium	40.0 b
	in lesioned apple	100.0 c
Mycelium	on uninjured apple	0 a
	on lesioned apple	100.0 c

P=0,05

Table 5

Effect of absence of particular components in Czapek's medium on *P. expansum* culture growth and infection of uninjured apples by *P. expansum* after 10 days at 20°C

Lacking component of medium	Degree of growth		Rotting apples after infection, %
	agar medium	liquid medium	
FeSO <sub>4</sub> · 7H <sub>2</sub> O	5	5	80
K <sub>2</sub> H · PO <sub>4</sub>	3	2	20
KCl	1	4	20
MgSO <sub>4</sub> · 7H <sub>2</sub> O	4	3	10
NaNO <sub>3</sub>	2	1	10
No sugar	3	1	0
Liquid component of medium	5	5	100

5 — normal growth, no change of colour and sporulation;

4 — good growth, no change of colour, numerous coremia formed;

3 — poor growth, colour lighter, diffuse, only centre normally coloured;

2 — very poor growth, colour white, diffuse, centre green;

1 — culture very poor, white, diffuse.

no proved intensification of fruit rotting occurred when the skin was uninjured, even when the apples were placed on a cube of malt agar extract medium or on a cube of decaying apple. Somewhat better results were obtained when grated apple was used with fungus spores (17% of fruit decayed). If a piece of apple was used as substrate for the spores with developing *Gloeosporium perennans* (*Pezicula malicorticis*) the infected fruits rotted in 25 percent of the cases. Fruits with injured epidermis infected with dry spores all rotted.

Comparison of liquid Czapek's medium and the same solidified with agar showed a weaker influence of the medium with agar on the process of infection.

Mycelium placed on an uninjured apple did not cause decay.

The markedly positive influence of Czapek's liquid medium on infection of uninjured fruits required closer study. For this purpose growth of *P. expansum* was observed on Czapek's medium deprived of one component and compared with growth on the liquid and solid medium. The results are shown in Table 5. The fungus grew normally on medium lacking  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , as on the full medium. Exclusion of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  caused a greater difference in growth of the culture, particularly on liquid medium, whereas the absence of KCl considerably restricted growth on the agar medium and very little on liquid medium. The lack of sugar affected growth more on liquid than on agar medium. The influence of the composition of liquid medium and its stimulating effect on the virulence of *P. expansum* towards uninjured fruits was visible and corresponded to the growth of cultures on media deprived of certain components. Liquid medium without sucrose had an influence on infection of apples, the absence of hydrated iron sulphate had little effect, in this medium 80 per cent of the fruit rotted, but removal of hydrated magnesium sulphate and sodium nitrate resulted in a considerable decrease of the per cent of rotting fruits.

Table 6

Effect of components of solution used in inoculum on the ability of *P. expansum* to infect uninjured apples of the Glogierówka cv.

Inoculum components added to 3% sucrose	Rotting apples %	Inoculum without sucrose	Rotting apples %
0.5 g/l KCl	20	KCl	0
1 g/l $\text{K}_2\text{HPO}_4$	20	$\text{K}_2\text{HPO}_4$	0
0.01 g/l $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	30	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	10
0.5 g/l $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	60	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0
3 g/l $\text{NaNO}_3$	90	$\text{NaNO}_3$	0
Complete Czapek's medium	100	3% sucrose	10
Water	0		



Table 6 shows the influence of the particular components of Czapek's medium used in concentrations corresponding to their amount in the latter with 3 per cent sucrose added or in aqueous solution on infection of intact Glogierówka apples. It was proved that the particular components in the absence of sugar do not enhance rotting of uninjured fruits, whereas  $\text{NaNO}_3$  and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  with sugar have a strong effect. Sugar itself had no major effect on rotting.

#### IV. COEXISTENCE OF SPECIES OF THE GENUS *PENICILLIUM* ON ROTTEN FRUITS

During isolation of the fungi it was found that two or three species of the genus *Penicillium* occur frequently together in the mould spots.

Studies were performed to identify the coexisting species parasitizing the fruits.

#### Material and methods

During isolation of the fungi from the rotten fruits it was not possible to recognize whether one or several fungus species were present. The species could be identified only when one-spore cultures were isolated. Marking in detail each isolate and the cultures obtained from it made it possible to establish the coexistence of the particular species.

#### Results

The results showing the coexistence of the other studied species with *P. expansum* are listed in Table 7. The fungus was found to occur most frequently alone (77.8% isolation from apples and 66 from pears). In 14 per cent of isolates from apples and 19.5 percent from pears the fungus grew together with *P. diversum*, and only in 3 per cent of the

Table 7

Coexistence of three fungus species of the genus *Penicillium* with *P. expansum*. Per cent of occurrence is given in relation to number of isolates of *P. expansum*

Fungus species	Isolates from apples %	Isolates from pears %
<i>P. expansum</i> (alone)	77.8	66.7
<i>P. expansum</i> + <i>P. diversum</i>	14.1	19.5
<i>P. expansum</i> + <i>P. cyclopium</i>	3.3	9.4
<i>P. expansum</i> + <i>P. spinulosum</i>	3.0	1.3
<i>P. expansum</i> + <i>P. diversum</i> + <i>P. cyclopium</i>	0.5	1.3
<i>P. expansum</i> + <i>P. diversum</i> + <i>P. spinulosum</i>	1.4	1.9

Table 8

Coexistence of three fungus species of the genus *Penicillium* with *P. diversum*. Per cent of occurrence is given in relation to number of isolates of *P. diversum*

Fungus species	Isolates from apples %	Isolates from pears %
<i>P. diversum</i> (alone)	48.4	49.4
<i>P. diversum</i> + <i>P. expansum</i>	37.3	40.3
<i>P. diversum</i> + <i>P. cyclopium</i> + <i>P. expansum</i>	1.2	2.6
<i>P. diversum</i> + <i>P. expansum</i> + <i>P. spinulosum</i>	3.6	3.9
<i>P. diversum</i> + <i>P. cyclopium</i>	8.7	—
<i>P. diversum</i> + <i>P. spinulosum</i>	0.8	3.9

Table 9

Coexistence of three fungus species of the genus *Penicillium* with *P. cyclopium*. Per cent of occurrence is given in relation to the number of isolates of *P. cyclopium*

Fungus species	Isolates from apples %	Isolates from pears %
<i>P. cyclopium</i> (alone)	50.0	57.5
<i>P. cyclopium</i> + <i>P. expansum</i>	21.6	37.5
<i>P. cyclopium</i> + <i>P. expansum</i> + <i>P. diversum</i>	2.9	5.0
<i>P. cyclopium</i> + <i>P. diversum</i>	21.6	—
<i>P. cyclopium</i> + <i>P. spinulosum</i>	3.9	—

Table 10

Coexistence of three fungus species of the genus *Penicillium* with *P. spinulosum*. Per cent of occurrence is given in relation to the number of isolates of *P. spinulosum*

Fungus species	Isolates from apples %	Isolates from pears %
<i>P. spinulosum</i> (alone)	52.1	60.0
<i>P. spinulosum</i> + <i>P. expansum</i>	27.4	10.0
<i>P. spinulosum</i> + <i>P. diversum</i>	2.7	15.0
<i>P. spinulosum</i> + <i>P. cyclopium</i>	5.5	—
<i>P. spinulosum</i> + <i>P. expansum</i> + <i>P. diversum</i>	12.3	15.0

cases on apples and 9 per cent on pears the coexistence of *P. expansum* and *P. cyclopium* was noted. Still less frequent was the coexistence of *P. expansum* and *P. spinulosum* (3% on apples and 1.3% on pears). Sporadically 3 species were found parasitizing simultaneously on one mould spot.

Coexistence of other species with *P. diversum* is shown in Table 8. The latter was found separate only in 48.4 per cent of the isolates from apples and 49.4 per cent from pears. In the remaining cases it mostly occurred with *P. expansum*, and much less frequently (8.7%) with *P. cyclopium* on apples. With other species it only coexisted sporadically.

*P. cyclopium* in one half of the cases is found alone (Table 9). It coexists most frequently with *P. expansum* (21.6% of isolates from apples and 37.5% from pears). It was also found together with *P. diversum* (21.6% of isolates from apples). With the remaining species it only occurred sporadically.

*P. spinulosum* was found alone in 52.1 per cent of the isolates from apples and 60 per cent from pears (Table 10). It was found with *P. expansum* on apples (27%) and *P. diversum* or *P. diversum* and *P. expansum* in 15 per cent of the isolates from pears. These 3 species were quite frequently found on apples (12.3%).

## V. PATHOGENICITY OF THE DIFFERENT ISOLATES OF *PENICILLIUM* SPP.

### Material and methods

For establishing the pathogenicity of the particular isolates of each species under study, a number of artificial infections were performed. Ten injured apples in each combination were infected with a swab of sterile absorbent cotton dipped in an aqueous suspension of spores of the given fungus. The suspension was prepared in distilled sterile water. The fruits were stored in high humidity chamber (95%). After 10 days the number of rotting fruits was counted. Four classes of pathogenicity were established according to the number of successful inoculations:

- |     |       |    |               |  |
|-----|-------|----|---------------|--|
| I   | group | of | pathogenicity | — all sites of inoculation, rotting after 10 days. |
| II  | „     | „  | „             | — 3/4 of inoculation sites, rotting after 10 days. |
| III | „     | „  | „             | — 1/2 of inoculation sites, rotting after 10 days. |
| IV  | „     | „  | „             | — 1/4 of inoculation sites, rotting after 10 days. |

A total of 812 isolates were tested, that is 8120 infecting inoculations were performed. Apples of the Boiken cv. were infected.

Besides the isolates, 14 species the Mycological Institute at Kew, G. Britain, were tested: *P. brevi-compactum* Dierckx (17456), *P. funiculosum* Thom (87160), *P. chrysogenum* Thom (37767), *P. granulatum* Bainier (92218), *P. janthinellum* Biourge (90838), *P. cyclopium* Westling (92236), *P. spinulosum* Thom (92251), *P. viridicatum* Westling (91958), *P. oxalicum* Currie and Thom (112755), *P. variabile* Sopp (112201), *P. frequentans* Westling (91159), *P. nigricans* (Bainier) Thom (104603), *P. citrinum* Thom (61272), *P. roqueforti* Thom (92261). The pathogenicity of those species was tested by inoculating apples of the Cox Orange cv. and pears of the Williams cv.

### Results

The pathogenicity of the particular species is compared in Table 11. All the tested isolates are grouped according to the degree of their virulence towards the apples.

Table 11

Pathogenicity of isolates of a particular fungus towards apples. Per cent of isolates is given in each group of pathogenicity: group I includes most pathogenic and group IV least pathogenic isolates

Fungus species	Group of pathogenicity				Total no. of isolates tested
	I	II	III	IV	
<i>P. expansum</i>	91.97	3.90	3.04	1.09	461
<i>P. diversum</i>	86.12	6.70	2.87	4.31	209
<i>P. cyclopium</i>	87.14	8.57	2.86	1.42	70
<i>P. spinulosum</i>	88.89	5.56	2.78	2.78	72
Total					812

Table 12

Pathogenicity of tested species expressed as per cent of unsuccessful infections

Fungus species	Unsuccessful infections
	%
<i>P. expansum</i>	5.51 a
<i>P. diversum</i>	11.37 b
<i>P. spinulosum</i>	8.63 ab
<i>P. cyclopium</i>	10.53 b

Most pathogenic proved to be *P. expansum*. The remaining 3 species showed wide differences in virulence in dependence on the isolate. Besides highly pathogenic ones, isolates with low pathogenicity were most numerous in the case of *P. diversum*. Table 12 gives the percent of unsuccessful inoculations with the particular species. The smallest number of apples remained healthy in the case of inoculation with

a spore suspension of *P. expansum*, the remaining 3 species, *P. diversum*, *P. cyclopium* and *P. spinulosum* showed a similar pathogenicity towards apples.

The pathogenicity of the 14 species from England is shown in Table 13. To group I of pathogenicity towards apples belong the following species: *P. funiculosum*, *P. chrysogenum*, *P. viridicatum*, *P. variable*, *P. frequentans*, *P. citrinum*, and towards pears: *P. cyclopium*, *P. spinulosum*, *P. viridicatum*, *P. variable*, *P. citrinum*, *P. nigricans*, *P. roqueforti*; Least pathogenic to pears was *P. frequentans*, and to apples *P. granulatum*. The fact should, however, be stressed that all the tested species could have been apple pathogens in the period of storage.

Table 13

Pathogenicity of fungi of the genus *Penicillium* from the English collection (Mycological Institute, Kew, England)

Fungus species	Groups of pathogenicity towards	
	pears	apples
<i>P. brevicompactum</i> 17456	II	II
<i>P. funiculosum</i> 87160	II	I
<i>P. chrysogenum</i> 37767	II	I
<i>P. granulatum</i> 92218	II	IV
<i>P. janthinellum</i> 90838	II	II
<i>P. cyclopium</i> 92236	I	II
<i>P. spinulosum</i> 92251	I	IV
<i>P. viridicatum</i> 91958	I	I
<i>P. oxalicum</i> 112755	II	III
<i>P. variable</i> 112291	I	I
<i>P. frequentans</i> 91159	IV	I
<i>P. n. gracilis</i> 104603	I	II
<i>P. citrinum</i> 61272	I	I
<i>P. roqueforti</i> 92261	I	III
Pears Williams cv.		
Apples Cox Organe cv.		

### CONCLUSIONS

1. Wet rot of storage apples and pears may be caused by several species of fungi of the genus *Penicillium*. Most frequently it is *P. expansum* and less is *P. diversum*, *P. cyclopium* and *P. spinulosum* occur rather seldom.

2. The main source of infection of the fruits are spores from the air in the orchard, normal storage, or cold storage. Only the species *P. diversum* and *P. spinulosum* were isolated from the soil. The bark surface in the tree crowns was not a source of infection. Since spores

of fungi of the genus *Penicillium* are generally present in the air, disinfection of the storage rooms is of no major importance as treatment the occurrence of *P. expansum*.

3. Apples with uninjured skin can be infected by *P. expansum* spores if they have access to some nutrients. The course of infection with *P. expansum* is stimulated by metabolites formed in the medium during spore germination of *Pezizula malicorticis*, the cause of bitter apple rot. Metabolites of germinating spores of *Botrytis cinerea* are not influence the *P. expansum* infection. The most important components of the medium stimulating infection with *P. expansum* of intact fruits are nitrogen compounds, particularly  $\text{NaNO}_3$  in the presence of sucrose. A stimulating effect of magnesium salt was also observed.

4. Species of the genus *Penicillium* frequently occur together on the same mould spot. The most frequent species appearing alone is *P. expansum*. In most cases of wet apple rot of storage coexistence of two species was found.

5. Wet apple rot in storage may be caused by the species: *P. expansum*, *P. diversum*, *P. cyclopium* and *P. spinulosum*, but *P. expansum* is most pathogenic. The remaining species show wide differences in the pathogenicity of the particular isolates.

Pears are more susceptible than apples to infection by fungi of the genus *Penicillium* during the storage period.

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## Grzyby z rodzaju *Penicillium* występujące na jabłkach i gruszkach w okresie przechowywania

### Streszczenie

Duże znaczenie gospodarcze zgnilizn przechowywanych owoców stworzyło konieczność poznania etiologii i patogenezы poszczególnych czynników chorobotwórczych.

Głównymi sprawcami mokrej zgnilizny owoców ziarnkowych okazały się grzyby z rodzaju *Penicillium* i to nie jeden gatunek, jak dotychczas sądzono, ale cztery gatunki: *P. expansum* Link., *P. diversum* Raper et Fennel, *P. cyclopium* Westing i *P. spinulosum* Thom.

Częstotliwość występowania poszczególnych gatunków ustalono na podstawie oznaczenia 1391 izolatów pochodzących z 17 różnych rejonów Polski, w czym 1095 izolatów pochodziło z gnijących jabłek, a 296 z gnijących gruszek.

Najliczniej reprezentowany był gatunek *P. expansum*, on też wykazał najsilniejszą patogeniczność w stosunku do jabłek. Pozostałe gatunki okazały się bardzo zróżnicowane pod względem patogeniczności w zależności od izolatu. Na podstawie przeprowadzonych sztucznych zakażeń piętnastoma gatunkami grzybów z rodzaju *Penicillium* stwierdzono, że prawie każdy gatunek grzyba z tego rodzaju może spowodować zgniliznę uszkodzonych jabłek lub gruszek.

Na jednej plamie gnilnej stwierdzono występowanie kilku gatunków grzybów z rodzaju *Penicillium*. *P. expansum* w 78%, *P. diversum* w 48%, *P. cyclopium* w 50%, a *P. spinulosum* w 52% przypadków występowały pojedynczo, w pozostałych przypadkach stwierdzono ich współistnienie z innymi gatunkami tego samego rodzaju.

W badaniach dotyczących procesu infekcji przez grzyb *P. expansum* stwierdzono, że owoce o nie uszkodzonej skórce mogą być zakażane przez zarodniki tego grzyba jedynie w przypadku, gdy zostanie zapewnione dodatkowe źródło składników pokarmowych, takich jak cukier i azot, głównie w postaci  $\text{NaNO}_3$ .

W ramach poszukiwania źródła zakażenia stwierdzono, że zarodniki unoszące się w powietrzu odgrywają podstawową rolę w infekcji owoców.