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The pathogenicity of different *Botrytis cinerea* Pers. isolates to apples and their sensitivity to benzimidazole fungicides

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Abstract

The pathogenicity of 80 isolates of *Botrytis cinerea* Pers. from different hosts to apple fruit was examined. Host specificity among isolates was not found. All of the isolates, independent of their derivation, caused apple fruit rot. Isolates from apple fruits showed moderate and strong pathogenicity to apple fruits. Only 1 of the 22 examined isolates showed weak pathogenicity. Tolerance to benomyl was compared among isolates obtained from apple fruits and from other hosts. It was found that 35% of isolates from apples showed resistance to benomyl. There was no correlation between the pathogenicity of isolates and their resistance to benomyl.

INTRODUCTION

The fungus *Botrytis cinerea* Pers. (perfect stage *Botryotinia fuckeliana* (de Bary/Whetzel) is a typical polyphagous fungus. It is also the cause of apple rot during storage.

There is no uniform opinion in literature of the parasitic specialization of *Botrytis cinerea* Pers. M a r r a s (1960) and D u b o s and B u l i t (1973) found a certain kind of parasitic specialization. The fungus isolated from the grapevine was more pathogenic to grapevines than that isolated from other plants. M a c N e i l (1953) observed an unusual case of pathogenic specialization of *Botrytis cinerea* Pers. in respect to organs of the same plant. An isolate from lettuce was extremely pathogenic to lettuce roots and very weakly pathogenic to its foliage. Differentiation of pathogenicity of *Botrytis cinerea* Pers., in respect to the tomato was demonstrated by I l e v a (1977). On the other hand however, S c h n e l l h a r d t and H e a l d (1936), P e y e r (1963) and S a d o w s k i (1971) did not find differences in the pathogenicity of *Botrytis cinerea* isolated from different plants.

Aside from differences in pathogenicity of the fungus, the formation of strains reacting differently to fungicides is also of great significance. The most common

cases of resistance of *Botrytis cinerea* Pers. concern benzimidazole fungicides. In the years 1972-73 in Poland, sporadic cases of resistance on raspberries and strawberries were observed, in later years, resistant forms of the fungus were found very often on different plants (A r s e n i u k and B r y k, 1978). There is very little information in literature on resistant forms of this fungus found on apples. G r i m m (1977) and G j a e r u m (1978) isolated strains of this fungus resistant to benzimidazoles from rotten apples whereas B e r t r a n d and S a u l i e-C a r t e r (1978) isolated resistant strains from the water in the flotation dump tanks. Benzimidazole fungicides are still advised to be used before harvest of apples because they are effective in control of *Pezicula* sp. which also cause apple rot during storage (B o r e c k a and C e g ł o w s k a, 1973).

The aim of the study presented here was to evaluate the pathogenicity of *Botrytis cinerea* Pers. isolates to apples in order to find if apples are attacked by a specialized form of the fungus. The reaction to benzimidazole fungicides by different isolates of *Botrytis cinerea* Pers. among which were some from apples, was also determined. The resistance of the fungus is especially dangerous when it coincides with its strong pathogenicity. For this reason, in these studies it was attempted to check if there is a correlation between the resistance of *Botrytis cinerea* Pers. isolates to benzimidazoles and their pathogenicity.

MATERIALS AND METHODS

The experiments were conducted on 80 *Botrytis cinerea* Pers. isolates from different plants and the air in a cold-storage chamber. The origin of the individual isolates is given in Table 1. After isolation and identification of the fungus, all of the isolates were maintained in test tubes on PDA (Difco). The pathogenicity of the fungus was tested by artificial inoculations of apples of the 'Cortland' variety which were ripe for consumption. The apple skin was incised with a sterile scalpel. A disk of the mycelium, 5 mm in diameter, cut from a 5 day-old culture of the fungus on PDA, was applied in the incision. The place of inoculation was protected with foil. The inoculated apples were placed under conditions favorable for development of the fungus, that is, at a temperature of 20°C and 90% relative humidity. Twenty apples were inoculated with each isolate in two places, giving thus 40 inoculation points. The apples were uniform in size. After 4 days of incubation, the diameter and depth of the rot were measured. The volume of the rotted apple tissue, which was more or less conical in shape, served to indicate the degree of pathogenicity. The results were analysed statistically by the variance method, averages were compared using the Duncan Test.

The susceptibility of *Botrytis cinerea* Pers. to benzimidazole fungicides was studied using 111 isolates, among which were the 80 tested for pathogenicity. The origin of the remaining 31 isolates are given in Table 2. The isolates were gathered

Table 1

The hosts and pathogenicity to apples, expressed as the volume of rotting apple tissue, of various *Botrytis cinerea* Pers. isolates

Isolate no.	Host	Volume of rotting tissue in cm^3
1	2	3
33	raspberries	0.09 a
59	strawberries	0.12 b
2	strawberries	0.21 c
37	raspberries	0.33 d
38	raspberries	0.41 de
48	tomato	0.52 e
26	raspberries	0.53 e
35	raspberries	0.59 ef
50	tomato	0.66 ef
4	strawberries	0.66 ef
63	blueberry	0.67 ef
7	raspberries	0.77 f
3	raspberries	0.81 f
34	raspberries	0.82 f
40	raspberries	1.10 g
11	peach	1.11 g
25	raspberries	1.14 g
29	raspberries	1.20 g
55	apple	1.27 gh
27	raspberries	1.43 gh
16	strawberries	1.64 h
5	raspberries	1.65 h
56	pear	1.67 h
36	raspberries	1.78 hi
68	apple	2.16 hi
20	raspberries	2.16 hi
61	apple	2.18 hi
6	raspberries	2.18 hi
69	apple	2.21 hi
21	raspberries	2.33 i
60	apple	2.37 i
24	raspberries	2.44 i
42	Saintpaulia	2.68 ij
66	blueberries	2.69 ij
39	raspberries	2.73 ij
43	raspberries	2.78 ij
52	carnation	2.85 ij
14	strawberries	2.86 ij
77	apple	2.86 ij
12	paprika	2.90 ij
72	apple	2.90 ij

Table 1 cont.

1	2	3
15	strawberries	3.03 ij
10	cauliflower	3.04 ij
28	raspberries	3.05 ij
31	tulip	3.08 ij
65	blueberries	3.16 ij
41	raspberries	3.23 ij
53	pear	3.48 j
57	apple tree	3.53 jk
30	raspberries	3.63 jk
70	apple	3.70 jk
71	apple	3.76 jk
79	apple	3.88 jk
80	apple	3.91 jk
64	blueberries	3.92 jk
1	strawberries	3.97 jk
46	apple	4.02 jk
58	rose	4.16 jk
74	air from a cold storage chamber	4.18 jk
8	onion	4.18 jk
51	pear	4.24 jk
19	raspberries	4.31 jk
17	raspberries	4.47 jk
32	raspberries	4.92 k
13	apple	5.22 k
78	air from a cold storage chamber	5.30 k
67	chrysanthemum	5.39 k
9	tomato	5.41 k
44	apple	5.44 k
23	raspberries	5.45 k
49	apple	5.58 k
47	apple	5.78 kl
62	apple	5.80 kl
75	apple	6.04 kl
76	apple	6.26 kl
18	raspberries	6.36 kl
45	apple	6.37 kl
73	apple	6.49 kl
22	raspberries	6.53 kl
54	apple	7.97 l

P = 0.05

taking into account the diversity of the hosts, not the intensity of chemical protection used. All of the isolates were cultured on PDA medium, then tested by poisoning the medium. One fungicide from the benzimidazole group was used in these studies since it has been determined many times that *Botrytis cinerea*

resistance to these fungicides is of a cross-resistance type for the whole group of benzimidazole compounds. The fungicide as product (Benlate 50 WP) was added to PDA (Difco) after its sterilization. The fungicide dose was determined by calculating the amount of active ingredient. Three fungicide concentrations were used: 1 ppm, 10 ppm and 100 ppm. The control was medium without fungicide. These concentrations of fungicide allowed the studied isolates to be classified into one of the four groups of sensitivity to fungicides established by B o l l e n and F u c h s (1970):

ED_{50} > 100 ppm	–	resistant fungi
ED_{50} from 10 to 100 ppm	–	tolerant fungi
ED_{50} from 1 to 10 ppm	–	sensitive fungi
ED_{50} < 1 ppm	–	very sensitive fungi.

In respect to resistant isolates, two additional concentrations of the fungicide were used – 1000 ppm and 10 000 ppm. After inoculation of the fungus onto the medium with fungicide and control without fungicide, the dishes were placed at a temperature of 20°C. Each combination was composed of 5 replications. The diameter of the mycelium was measured after 3-5 days when the control mycelium's diameter was 70 mm.

In order to find if the reaction of *Botrytis cinerea* Pers. to benzimidazoles is connected with its pathogenicity, the percent of isolates waekly, moderately and strongly pathogenic to apples was calculated for each group of sensitivity to benzimidazole fungicides.

T a b l e 2

The hosts of the remaining 31 *Botrytis cinerea* Pers. isolates studied for sensitivity to benomyl

Host	Isolate no.
Apple	81, 82, 84, 85, 86, 91, 98, 99, 100, 101, 102, 103, 105, 106, 107, 108, 109, 110
Air in cold storage chamber	83, 96, 97
Blueberry	87, 92
Bean	88
Black currant	89
Mallow	90
Sweet cherry	93
Plum	94
Grape	95
Strawberry	104
Kale	111

RESULTS

The pathogenicity of 80 studied *Botrytis cinerea* Pers. isolates is presented in Table 1. All of the isolates were pathogenic to 'Cortland' variety apples; in all cases, apple rot took place after inoculation. The isolates differed greatly in their degree of pathogenicity. Statistical analysis discerned 12 classes of significance. The least pathogenic isolate (no. 33) caused 0.09 cm^3 of rot whereas the most pathogenic one (no. 54), 7.97 cm^3. The pathogenicity of the isolate was not connected with its origin. Isolate from different plants were among both weakly and strongly pathogenic forms.

The isolates were divided into two groups on the basis of their origin, that is, from apples and from other hosts. In order to compare the pathogenicity of apple isolates with the others, three classes of pathogenicity were established:

class	volume of rotted tissue	pathogenicity
I	0 – 2 cm^3	weak
II	2.1 – 5.0 cm^3	moderate
III	5.1 – 8.0 cm^3	strong

Next, the percentage of isolates belonging to each of the three classes of pathogenicity was calculated in the group of isolates from apples and other hosts (Table 3). A slightly greater pathogenicity of apple isolates was found. Most of the isolates derived from apples belonged to the II and III classes of pathogenicity, only 4.5% to class I. The isolates from other hosts belonged mainly to classes I and II with only 10.3% belonging to class III.

The susceptibility of the studied *Botrytis cinerea* Pers. isolates to benomyl was diverse (Tables 4 and 5). According to the Bollen and Fuchs classification, among the 111 isolates, 67 very sensitive (60.4%), 3 sensitive (2.7%), 1 tolerant (0.9%) and 40 resistant (36.0%) isolates were found. Most of the isolates were either sensitive

Table 3

Comparison of the pathogenicity to apples of *Botrytis cinerea* Pers. isolates from apples and from other hosts

Isolates	Isolates in pathogenicity class (%)		
	I (0 - 2.0 cm^3)	II (2.1 - 5.0 cm^3)	III (5.1 - 8.0 cm^3)
From apples	4.5	50.0	45.5
From other hosts	39.7	50.0	10.3

Table 4

Susceptibility to benomyl of *Botrytis cinerea* Pers. isolates from apples

Isolate no.	Diameter of culture (in mm) on PDA medium containing benomyl in the following concentrations				
	1 ppm	10 ppm	100 ppm	1000 ppm	10000 ppm
13	0.0	0.0	0.0	–	–
44	0.0	0.0	0.0	–	–
45	0.0	0.0	0.0	–	–
46	0.0	0.0	0.0	–	–
47	0.0	0.0	0.0	–	–
49	15.0	0.0	0.0	–	–
54	14.0	0.0	0.0	–	–
55	0.0	0.0	0.0	–	–
60	9.0	0.0	0.0	–	–
61	70.0	70.0	70.0	44.1	0.0
62	70.0	70.0	70.0	43.6	11.4
68	0.0	0.0	0.0	–	–
69	70.0	70.0	70.0	44.8	0.0
70	0.0	0.0	0.0	–	–
71	31.1	12.4	0.0	–	–
72	0.0	0.0	0.0	–	–
73	70.0	70.0	70.0	32.0	0.0
75	70.0	70.0	70.0	31.3	0.0
76	23.0	21.5	0.0	–	–
77	70.0	70.0	70.0	32.4	0.0
79	70.0	70.0	70.0	25.3	0.0
80	70.0	64.0	55.4	0.0	0.0
81	70.0	70.0	70.0	29.0	0.0
82	10.3	0.0	0.0	–	–
84	70.0	70.0	70.0	32.0	0.0
85	70.0	70.0	41.3	0.0	0.0
86	70.0	70.0	70.0	27.8	0.0
91	60.4	56.0	21.9	0.0	0.0
98	0.0	0.0	0.0	–	–
99	0.0	0.0	0.0	–	–
100	70.0	70.0	70.0	23.4	0.0
101	0.0	0.0	0.0	–	–
102	0.0	0.0	0.0	–	–
103	0.0	0.0	0.0	–	–
105	9.9	0.0	0.0	–	–
106	0.0	0.0	0.0	–	–
107	70.0	70.0	70.0	0.0	–
108	20.5	0.0	0.0	–	–
109	0.0	0.0	0.0	–	–
110	70.0	15.9	0.0	–	–

Table 5

Susceptibility to benomyl of *Botrytis cinerea* Pers. isolates from different hosts

Isolate no.	Diameter of culture (in mm) of PDA medium containing benomyl in the following concentrations				
	1 ppm	10 ppm	100 ppm	1000 ppm	10000 ppm
1	2	3	4	5	6
1	70.0	70.0	54.4	6.7	0.0
2	0.0	0.0	0.0	–	–
3	0.0	0.0	0.0	–	–
4	0.0	0.0	0.0	–	–
5	0.0	0.0	0.0	–	–
6	70.0	70.0	70.0	21.7	0.0
7	0.0	0.0	0.0	–	–
8	70.0	70.0	65.5	0	–
9	0.0	0.0	0.0	–	–
10	0.0	0.0	0.0	–	–
11	0.0	0.0	0.0	–	–
12	0.0	0.0	0.0	–	–
14	0.0	0.0	0.0	–	–
15	0.0	0.0	0.0	–	–
16	0.0	0.0	0.0	–	–
17	70.0	70.0	57.1	32.2	0.0
18	70.0	70.0	70.0	0	0.0
19	70.0	70.0	70.0	16.4	0.0
20	70.0	70.0	58.7	8.6	0.0
21	70.0	70.0	57.5	24.0	0.0
22	70.0	70.0	70.0	40.2	0.0
23	70.0	70.0	70.0	31.7	0.0
24	70.0	70.0	70.0	10.6	0.0
25	0.0	0.0	0.0	–	–
26	70.0	70.0	57.2	22.5	0.0
27	70.0	68.0	61.9	8.3	0.0
28	0.0	0.0	0.0	–	–
29	70.0	70.0	70.0	0.0	0.0
30	0.0	0.0	0.0	–	–
31	70.0	70.0	56.1	0.0	0.0
32	0.0	0.0	0.0	–	–
33	70.0	70.0	58.3	0.0	0.0
34	40.1	13.7	0.0	–	–
35	15.5	0.0	0.0	–	–
36	0.0	0.0	0.0	–	–
37	70.0	70.0	51.3	8.5	0.0
38	70.0	70.0	60.1	0.0	0.0
39	70.0	26.5	0.0	–	–
40	70.0	70.0	70.0	0.0	0.0

Table 5 cont.

1	2	3	4	5	6
41	70.0	70.0	70.0	9.8	0.0
42	25.2	0.0	0.0	–	–
43	0.0	0.0	0.0	–	–
48	70.0	70.0	70.0	37.1	0.0
50	70.0	70.0	70.0	0.0	0.0
51	0.0	0.0	0.0	–	–
52	0.0	0.0	0.0	–	–
53	0.0	0.0	0.0	–	–
56	0.0	0.0	0.0	–	–
57	0.0	0.0	0.0	–	–
58	70.0	70.0	70.0	0.0	0.0
59	0.0	0.0	0.0	–	–
63	0.0	0.0	0.0	–	–
64	0.0	0.0	0.0	–	–
65	0.0	0.0	0.0	–	–
66	0.0	0.0	0.0	–	–
67	0.0	0.0	0.0	–	–
74	70.0	70.0	70.0	42.0	0.0
78	70.0	70.0	70.0	18.0	0.0
83	0.0	0.0	0.0	–	–
87	0.0	0.0	0.0	–	–
88	13.5	0.0	0.0	–	–
89	0.0	0.0	0.0	–	–
90	0.0	0.0	0.0	–	–
92	0.0	0.0	0.0	–	–
93	0.0	0.0	0.0	–	–
94	0.0	0.0	0.0	–	–
95	0.0	0.0	0.0	–	–
96	0.0	0.0	0.0	–	–
97	0.0	0.0	0.0	–	–
104	0.0	0.0	0.0	–	–
111	70.0	70.0	61.6	30.5	0.0

Table 6

Comparison of the sensitivity to benomyl of *Botrytis cinerea* Pers. isolates from apples with that of isolates from other hosts

Sensitivity to benomyl class	Isolates from apples (40 isolates)	Isolates from other hosts (71 isolates)
I (very sensitive)	60.0%	60.6%
II (sensitive)	2.5%	2.8%
III (tolerant)	2.5%	0.0%
IV (resistant)	35.0%	36.6%

or resistant, intermediate forms were few. Six isolates exhibited a very high degree of resistance, ED_{50} for them exceeded 1 000 ppm benomyl.

A comparison of the sensitivity to benomyl of isolates from apples with the remaining ones is presented in Table 6. Both in the group of isolates from apples and that from other hosts, the proportion between resistant and sensitive to benomyl isolates was similar. In both groups, approximately 1/3 of the isolates were resistant.

The pathogenicity of 80 isolates to apples was compared with their reaction to benomyl, and the percenage of isolates of different pathogenicity in the classes very sensitive and resistant to benomyl, was calculated. The II and III class of sensitivity to benomyl were not taken into account because no isolate was found in the class III and only 2 isolates were found in class II. It was found that resistance to benomyl was not correlated with a greater pathogenicity of the fungus (Table 7). Highly pathogenic forms made up 21.2% of those isolates resistant to benomyl and 20.0% of those very sensitive to it. In both groups, almost half of the isolates were of moderate pathogenicity.

Table 7

Comparison of the pathogenicity of *Botrytis cinerea* Pers. to apples with its sensitivity to benzimidazole fungicides

Sensitivity to benomyl class	Isolates in pathogenicity class (%)		
	I (0 - 2.0 cm^3)	II (2.1 - 5.0 cm^3)	III (5.1 - 8.0 cm^3)
I – very sensitive isolates	31.1	48.9	20.0
II – resistant isolates	27.3	51.5	21.2

DISCUSSION

The variability of the fungus, *Botrytis cinerea* Pers., has been the subject of study for many years, however, a uniform opinion on the parasitic specialization of this fungus has not been reached yet. Some authors have found parasitic specialization to some degree (D u b o s and B u l i t, 1973), in the opinion of others (S c h n e l l h a r d t and H e a l d, 1936) no such specialization exists.

In this study it has been found that isolates from apples exbihited moderate or strong pathogenicity to apples. However, a weakly pathogenic apple isolate – no. 55 – belonging to class I of pathogenicity was found. This does not allow the thesis on parasitic specialization of the fungus to be confirmed.

All of the isolates were pathogenic to apples; regardless of its origin, the fungus caused apple rot. Since the *Botrytis cinerea* Pers. is a very common species the high possibility of apples infections may be expected.

A very high degree of variability of pathogenicity of the fungus to apples was shown. This is probably caused by different enzymatic activity of the individual isolates. W a s f y et al. (1978) and D i L e n n a et al. (1981) found a clear correlation between the pathogenicity of an isolate and the production of enzymes by it, mainly of polygalacturonase which plays a role in the maceration of apple tissue.

As a result of the studies conducted here, it was found that the susceptibility of *Botrytis cinerea* Pers. to benomyl. and therefore to the whole group of benzimidazole fungicides, is very differentiated. From the 111 isolates, 36% were resistant, 60.4% were very sensitive. Among the *Botrytis cinerea* Pers. isolates from apples, 35% were resistant. One isolate showed an extremely high degree of resistance – it even grew on a medium containing 10 000 ppm benomyl. Finding that only 35% of the isolates from apples are resistant indicates that, in spite of continuous use of these fungicides, the entire *Botrytis cinerea* Pers. population on apples has not become resistant.

The correlation of resistance to fungicides with a high pathogenicity of the fungus would create a dangerous situation for stored fruit because forms such as these could gain dominance in the population and eliminate the sensitive forms of the fungus. In the studies presented here, it has been shown that the pathogenicity of the isolate is not correlated with its reaction to benomyl. A similar observation had been made by B e r t r a n d and S a u l i e-C a r t e r (1978).

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Patogeniczność różnych izolatów *Botrytis cinerea* Pers. w stosunku do jabłek i ich reakcja na fungicydy benzimidazolowe

Streszczenie

Badano patogeniczność 80 izolatów *Botrytis cinerea* Pers., wyizolowanych z różnych gospodarzy, w stosunku do jabłek. Nie stwierdzono specjalizacji pasożytnictwa grzyba. Wszystkie izolaty, niezależnie od swojego pochodzenia, powodowały gnicie jabłek. Patogeniczność izolatów była bardzo zróżnicowana. Izolaty pochodzące z jabłek były średnio i silnie patogeniczne dla jabłek, tylko 1 izolat (na 22 badane) był słabo patogeniczny.

Badano reakcję na benomyl izolatów pochodzących z jabłek i z innych gospodarzy. 35% izolatów pochodzących z jabłek było odpornych na benomyl. Nie stwierdzono związku między patogenicznością izolatu a jego reakcją na benomyl.