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Occurrence and characteristics of Fusarium oxysporum f.sp.dianthi (Prill et Del.) Snyder et Hansen strains resistant to systemic fungicides

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Abstract

Strains of *Fusarium oxysporum* f. sp. *dianthi* (Prill et Del.) Snyder et Hansen, highly resistant to benomyl (Benlate), carbendazim — MBC (Bavistin) and methylthiophanate (Topsin M) were observed for the first time. They grew well in the presence up to 1000 ppm of MBC. The resistant strains usually grow considerably slower than the sensitive ones on unpoisoned media. Sporadic cases were noted of increasing transitional adaptative tolerance to MBC, after several transfers of sensitive strains onto media with low concentrations of the fungicide. An increasing frequency of resistant strains was observed under production conditions. Of the fungitoxicants used, MBC was more active towards the sensitive strains while the resistant strains tolerated considerably lower concentrations of benomyl than that of MBC.

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *dianthi* (Prill et Del.) Snyder et Hansen is the most dangerous disease of carnations. Protection consists in the production of healthy young plants and disinfection of the substrate. The introduction of systemic fungicides to which excessive hopes were attached, reduced the attention devoted to prophylactic measures, causing the sread of *Fusarium* wilt on greenhouse carnations in Poland.

In experiments on chemical control of *Fusarium* wilt on carnations in industrial greenhouses the treatments were found to be inffective in some cases owing to — as found out — the resistance of the fungus to systemic fungicides (among them to Benlate, Topsin M). Lately these cases are becoming more and more frequent.

The occurence nad characteristic of strains *of F. oxysporum* f. sp. *dianthi* highly resistant to these fungicides, are the subject of the

present paper. Such high resistance in this fungus has so far not been reported in the literature.

LITERATURE REVIEW

Among modern systemic fungicides most important are at present products of the group of benzimidazole and methylthiophanate. They have a similar scope and mechanism of action via similar products of their hydrolysis or transformation in the plant (Clemons and Sisler 1969; Bollen and Fusch 1970; Fuchs et al. 1974; Selling et al. 1971; Bollen 1972). A characteristic of these fungicides is given in review papers (Dekker 1972; Erwin 1973; Frahm 1973; Fuchs et al. 1974).

Soon after starting the application of systemic fungicides in plant protection, tolerant forms of the fungi appeared. The development of such forms among common and dangerous pathogens is becoming an economic problem. Since 1969 when the appearance of *Sphaerotheca fuliginea* strains resistant to benomyl on cucumbers was described (Schroeder and Provvidenti 1969), similar observations have been made in the case of a many other fungi. This has been discussed in a number of review papers (Dekker 1972; Erwin 1973; Frahm 1973; Fehrmann 1976). The adaptive tolerance of fungi towards the "traditional" fungicides was as a rule resticted in its scope and usualy did not influence the effectiveness of protection (review — Ashida 1965; Georgopoulos and Zaracovitis 1967). It is believed that the relatively rapid development in fungi of resistance to systemic fungicides may be connected with the mechanism of their action (Kaars-Sijpesteijn 1970; Clemons and Sisler 1971), and their high selective pressure (Dekker 1971). Benomyl causes disturbances of mitosis (Hastie 1971; Hammerschalg and Sisler 1973; Davidse 1973) and probably even has a mutagenice effect (Dassenoy and Meyer 1973). In some cases resistant forms appeared already after several treatments with the fungicide (Magie and Wilfred 1974; Littrell 1974).

Strains resistant to benomyl are usually resistant to the remaining fungicides of this group, although for instance resistance to benomyl and thiabendazol may be inherited independently (Hastie and Georgopoulos 1971).

Of greater practical importance may be the appearance of high resistance in *Botrytis cinerea* (among other authors Bollen and Scholten 1971, 1972; Jordan and Richmond 1974; Miller and Fletcher 1974), in the genus *Penicillinum* (Harding 1974; Bollen et al. 1974), in *Cercospora beticola* (Georgopoulos and Do-

vos 1973; Ruppel and Scott 1974), in *Venturia inaequalis* (Wicks 1974), Borecki (personel information) and several others.

Particularly interresting is the case of high resistance of a *Verticillium malthousei* strain isolated in 1958, thus before the invention of systemic fungicides (Wuest et al. 1974). This strain was, moreover, more sensitive than others to zineb (Lambert and Wuest 1975).

Strains with lowered sensitivity or tolerance to systemic fungicides were also obtained in the laboratory by way of transfers to increasing fungicide concentrations in the medium (Thanassoulopoulos et al. 1971; Luc et al. 1971; Richardson 1973; Abelensiev 1973; Kataria and Grover 1974; Benoit 1974; Waren et al. 1974). In many cases low tolerance is of reversible character. Similarly a very high and durable resistance of *Fusarium oxysporum* f. sp. *tulipae* (Valášková 1973) and f. sp. *melonis* (Dassenoy and Meyer 1973) was obtained.

The appearance of high resistance is of the nature of mutation. In *Fusarium oxysporum* f. sp. *melonis* natural mutants resistant to benomyl appear with a frequency of 1 in 8.6×10^7 spores (Bartels-Schooley and Mac Neill 1971). UV-induced mutants resistant to bezimidazole fungicides were also obtained (Ben-Yephet et al. 1974, 1975; Fourcade and Laville, 1973). Genetic investigations have been conducted on resistance to systemic fungicides (Georgopoulos 1971; Borck and Braymer 1974). In most cases resistant or tolerant strains grew poorly and their pathogenicity was weaker (Bollen and Scholten 1971; Georgopoulos et al. 1971; Wuest et al. 1974). Resistant strains of *Venturia inaequalis* (Wicks 1974), *Fusarium oxysporum* f. sp. *pisi* (Bochov et. al. 1971) preserve a high pathogenicity, in some cases resistant forms were even more pathogenic (Luc et al. 1974).

The forms of *Fusarium oxysporum* are highly sensitive to benomyl (Bollen and Fuchs 1970; Edginton et al. 1970). Richardson (1973) gives the ED_{50} value of benomyl as about 0.4-0.5 ppm for various forms of *F. oxysporum*. According to Valaskova (1973) *F. oxysporum* f. sp. *dianthi* is the most sensitive of all forms to benomyl.

Benzimidazole fungicides are highly effective against *F. oxysporum* on various plants, also on carnations (among other authors Pionat 1971; Baker 1972; Tramier and Antonini 1971, 1973). Tramier (1974), Tramier and Betachini (1974) noted, however, that *F. oxysporum* f. sp. *dianthi* may partly develop in the presence of 20 ppm MBC in carnations tissue, but isolates from these plants continued to be sensitive in vitro and was not changed by transfers on poisoned medium.

MATERIAL AND METHODS

From plants showing wilt symptoms (usually young 2-4 months after planting) the fungus was isolated by placing segments of shoots after superficial disinfection on dishes containing PDA medium with streptomycin sulphate (300 ppm) and PCNB (300 ppm) added, plus 1.5 and 100 ppm MBC (Bavistin 50 WP) or without the later. Isolates showing at least weak growth at 5 and 100 ppm concentrations of the fungicide were collected for further study or were immediately transferred to higher concentrations. Part of the isolates growing slowly in the presence of 5 ppm of MBC were transferred 2-4 times at 7-14-day intervals to medium with 5 ppm, and then gradually to higher concentrations. Concentrations of the fungicide, inhibiting growth on the medium were prelliminary determined. The range of sensitivity or resistance was preliminarily checked on cultures from mass isolations transferred successively (pices of fungus colonies), whereas comparison of chosen groups of isolates was done on one-spore strains. For inoculation of the dishes mycelium fragments from actively growing 5-10-day cultures were used.

Preliminary determinations of the range of resistance were performed at room temperature and final experiments at 24-25°C in darkness. Growth comparison of the chosen isolates was done on dishes 50 mm in diameter over the period when the standard sensitive strain on the medium without fungicide covered the dish (usually in 4-6 replications and preliminary observations in 2-3 replications). In the experiments commercial preparations of the fungicides were used; Benlate (50% benomyl), Bavistin (47% methylthiophanate), Funaben (50% carbendazim — MBC of Polish production).

The constancy of resistance of part of the strains was checked by transferring them for 12-18 months at 3-7-weeks intervale to fresh medium and checking their reaction to various fungicide concentrations. ED_{50} for sensitive, tolerant and resistant isolates was determined from the growth inhibition curve of one-spore strain cultures. Precise determination of the ED_{50} values for resistant isolates was not possible.

EXPERIMENTS AND RESULTS

The fungus was isolated at various times in the course of 4 years, as it appeared in various greenhouses in the environs of Warsaw (a total of 36 sites). In part of the cultures the young plants originated from different regions of the country or were imported, thus the review comprised a wide scope. In 4 greenhouses the samples were examined repeatedly.

Fungus forms highly resistant to MBC were detected with various

Table 1

Growth of several groups of isolates of *Fusarium oxysporum* f. sp. *dianthi* (Prill et Del.) Snyd. et Hansen from one greenhous complex on PDA with Bavistin (47% a.i. -MBC).

Fungus isolates	Relative growth of fungus colonies						
	Concentration of MBC (ppm)						
	0	0,5	1	5	100	500	1000
16	+++	+++	+	—			
11	++	++	+—	—			
31	+	+	+	++	+	+—	—
18	++	++	++	++	++	++	++
6	+++	+++	+	+—	—		
4	+	+	++	+	+—	+	+

*** Good, quick growth, diameter of colonies 41-50 mm;
** Intermediate rate of growth, colony diameter 25—40 mm;
* Slow growth, colony diameter 15—25 mm;
*— Very slow growth, colony diameter less than 15 mm.

frequency in 19 greenhouses. Observations of the latest months (not included in this study) showed an intensification of fusarium wilt even in new greenhouses, caused in several cases exclusively by resistant strains. The source of diseased plants were several farms producing young plants.

In one greenhouse complex exclusively producing carnations young plants (where in the previous years resistance of *Botrytis cinerea* to Benlate was very frequent) 165 samples were taken over 2.5 years from fusarium-diseased plants. Most fungus isolates from them were highly resistant to MBC. These isolates proved highly differentiated in their reaction to the fungicides. Numerous isolates resistant to 100-1000 ppm MBC, two tolerant to 5 ppm (weakly growing at 10 ppm) and strains of various sensitivity (ED_{50}=0.3-0.4 ppm MBC and 2 isolates with ED_{50}=0.5 ppm MBC) were obtained.

The classification of isolates from this farm is shown in Table 1.

Strains highly resistant to MBC could already be recognized during isolation. The mycelium growing out from the tissue showed good growth in the "poisoned" medium after several days. Sensitive isolates were limited in growth to the tissue from which they originated. In several cases, however, the fungus or mycelium growing from shoot segments became adapted after several days to the "poisoned" medium (5 ppm MBC) and slowly began to spread. From these sites the fungus was transferred to fresh media with a similar or somewhat higher MBC concentration. After 8-11 such transfers in 6 cases (of more than 40) the isolates obtained in this way grew quite well at a 5 ppm concentration of MBC (transfer of one-spore strains did not change in any of

the 16 cases their reaction to the fungicide). Four isolates (T-3, T-4, T-5 and T-6) transferred 9 times on 5 ppm and then 6 times on 10 ppm MBC were checked in comparative tests. The growth of these four isolates was normal like that of the sensitive ones on unpoisoned medium, and they even grew well in the presence of MBC and of the remaining fungicides (Tables 5, 6 and 7) showing ED_{50} 2-2.5 ppm MBC.

Three such tolerant isolates were retransferred on media without fungicides for 16 months (inoculated onto fresh medium at 4-6-week intervals) and were found to have lost much of their tolerance. They did not grow afterwards at all at 5 ppm and grew rather slowly at 1 ppm MBC (ED_{50} ca. 1 ppm). Some of the isolates resistant to 1000 ppm of MBC first grew very slowly after transfering to 500-1000 ppm and became more vigorous after further 2-4 transfers on this concentrations. Part of the isolates from plants which were known or suspected to be infected with resistant strains (progress of disease symptoms in spite of high Benlate or Topsin M doses) were transferred at once to medium with 100, 500 and 1000 ppm MBC. Two colonies growing from them showed normal growth on these media, comparable to that on medium without fungicide. A total of 216 resistant isolates were tested at various times on MBC concentrations up to 1000 ppm.

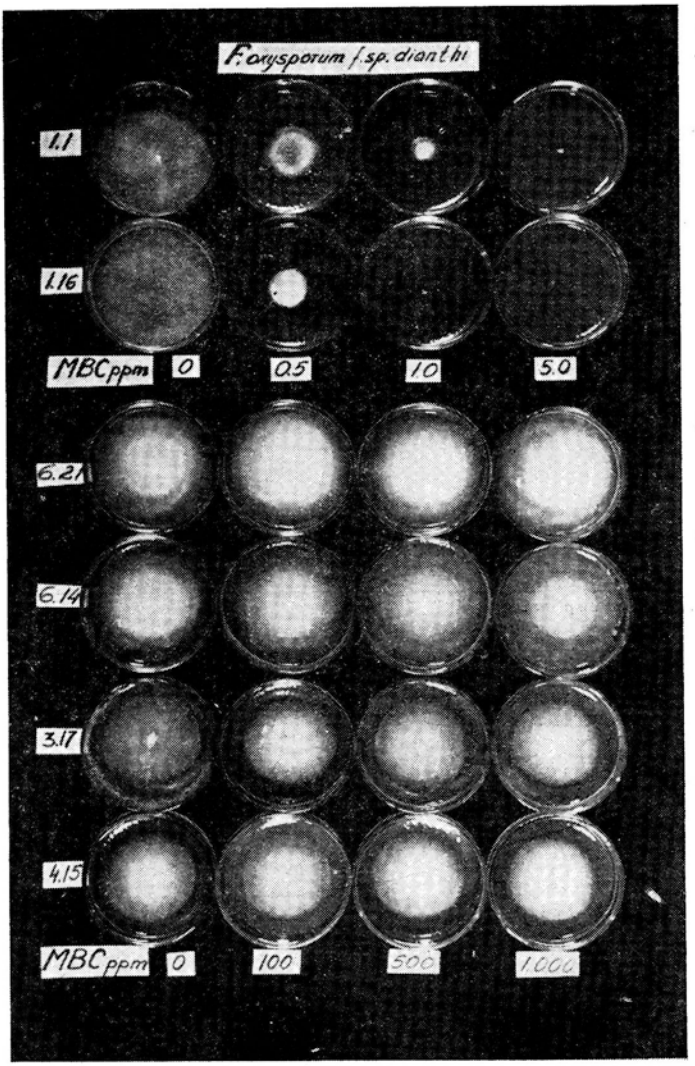
The preliminary tests with sensitive strains already showed small

Table 2

Influence of MBC (Bavistin, 47% a.i.) on mycelial growth of susceptible and resistant strains of *Fusarium oxysporum* f. sp. *dianthi* (Prill et Del.) Snyd. et Hansen

Fungus strains	Mean diameter of colonies in mm								
	Concentration of MBC (ppm)								
	0	0,5	1	5	25	100	500	1000	2000
1.1	50*	25	0	0					
1.16	50	16	4	0					
1.7	50	29	3	0					
1.31	50	17	0	0					
Fg-21	50	23	2	0					
Fg-29	48	26	3	0					
6.14	44				44	44	45	43	21
3.17	34				32	33	32	30	17
4.15	32				—	32	31	31	19
6.21	45				46	45	44	35	11
Fg-12	13				13	13	12	13	10
Fg-10	13				12	12	13	10	10
Fg-16	14				13	12	14	12	8
Fg-18	12				13	13	12	12	9
Fg-7	25				23	25	25	24	21

* Means of four replicates.



Phot. 1 Mycelial growth of *F. oxysporum* f. sp. *dianthi:* sensitive (1.1 and 1.16) and resistant strains on PDA with different concentrations of MBC. Differences within sensitive strains are visible at 1 ppm of MBC. Note differences in the rate of growth of resistant strains on poisoned PDA and also differences between sensitive and resistant strains in aerial mycelium development and colonies density

but distinct differences in the reaction to MBC. A large part of this isolates did not grow at all or very poorly in the presence of 1 ppm MBC in the medium, whereas others grew distinctly. The differences between two sensitive strains are shown on Photo 1 and in Tables 2, 3 and 7 (strains 1.16 and 1.1). This found confirmation in the small dif-

Table 3

Effect of benomyl and MBC on mycelial growth of resistant and susceptible strains *Fusarium oxysporum* f. sp. *dianthi* (Prill et Del.) Snyd. et Hansen

Fungus strains	Mean diameter of colonies in mm after 8 days						
	benomyl (ppm a.i.)				MBC (ppm a.i.)		
	0	0.5	1.0	5.0	0.5	1.0	5.0
Susceptible							
I	50*	38	15	0	31	6	0
1.16	50	36	6	0	18	0	0
1.12	50	25	12	0	20	3	0
1.1	50	26	14	0	17	4	0
	0	1,000	5,000	10,000	1000	5,000	10,000
Resistant							
XI	23	7	0	0	17	8	6
IX	43	15	0	0	29	9	5
6.14	21	16	0	0	21	10	4
3.16	40	8	0	0	24	11	6
6.2	23	6	2	0	22	13	8

* Means of four replicates.

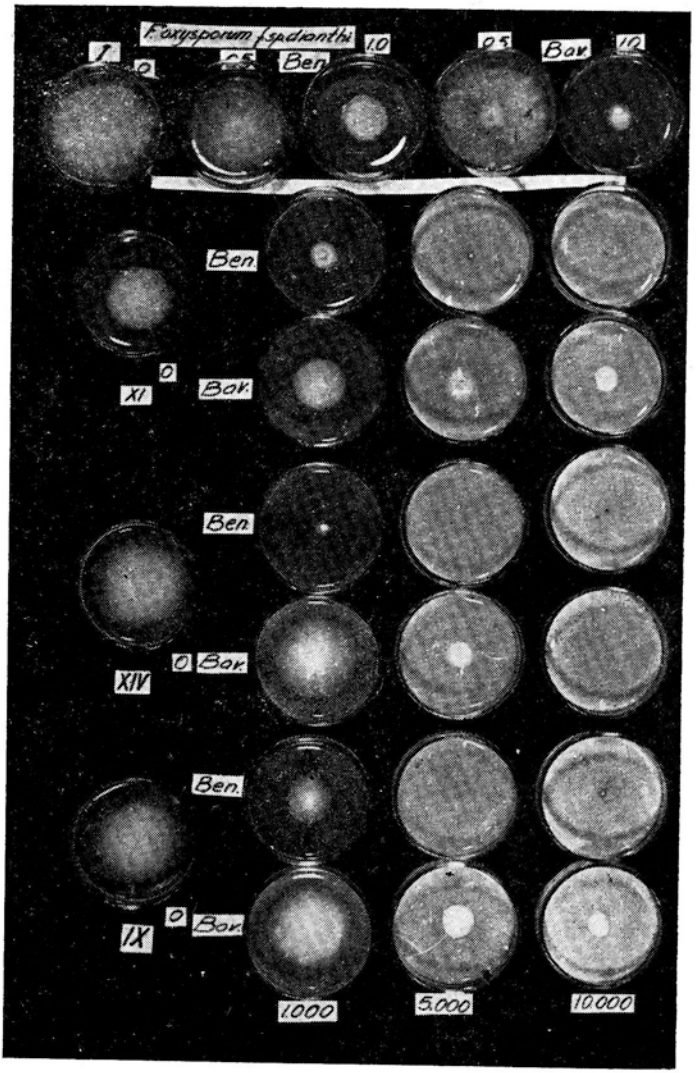
ference in the ED_{50} value (Table 6). ED_{50} for some of the isolates was 0.3 and for others 0.4 ppm and in two casess 0.5 ppm MBC. This fungicide proved distinctly omre active towards sensitive and tolerant isolates than benomyl (ED_{50} up to 0.6 ppm). Methylthiophanate (Topsin M) exhibited the lowest activity. Similar trends are illustrated by Tables 3 and 4 (upper part). Strains highly resistant to MBC did not grow equally without the fungicide nor at concentrations of up to 1000 ppm MBC, showing however, a marked growth depression at 5000 ppm. Most of these strains, grew slowly at concentrations as high as 10,000 ppm MBC. Only a small number of resistant isolates of this group did not grow at the highest fungicide concentrations (Phot. 2 and Tables 2, 3 and 4). Immediately after isolation of a series of strains from one greenhouse, isolates resistant to 100 and 500 ppm MBC did not grow at a 1000 ppm concentration (Table 1). From the group of 31 isolates after 3-4 transfers at 14-16-day intervals strains resistant could grow even up to 1000 and 5000 ppm MBC and two strains 6.14 and 6.2 still grew at 10,000 ppm (Tables 2 and 3). Thus, among the highly resistant forms there are possibilities of selection for further increase of resistance. It is interesting that 6 strains of this group, from wich one-spore cultures were prepared at once, did not change the resistance level in the course of 8-10 transfers in the presence of 1000, 2000 and 3000 ppm.

Table 4

Influence of benomyl (Benlate 50% a.i.), MBC (Bavistin 47% a.i.) and thiophate-methyl (Topsin M 70% a.i.) on mycelial growth of several isolates *Fusarium oxysporum* f. sp. *dianthi* (Prill et Del.) Snyd. et Hansen resistant and sensitive to MBC

Fungus isolates	Radial growth of colonies in mm after 8 days in 25°C															
	0	benomyl (ppm a.i.)					MBC (ppm a.i.)					thiophanate-methyl (ppm a.i.)				
		0.5		1,0		3.0	0.5		1.0		3.0	0.5		1.0		3.0
Sensitive																
1.1	50*	33		17		0	18		3		0	50		28		8
1.29	50	36		12		0	23		6		0	47		34		3
1.12	50	31		10		0	21		4		0	48		29		2
	0	100	500	1,000	5,000	1,0000	100	500	1,000	5.000	10,000	100	500	1,000	5,000	10,000
Resistant																
3.10	13	12	13	11	2	0	13	12	13	7	5	14	11	12	4	6
4.10	22	20	17	8	0	0	21	21	18	13	0	21	21	16	17	4
XIV	25	21	11	4	0	0	26	25	21	16	4	24	23	24	11	3
4.15	28	28	20	16	0	0	27	26	28	12	7	29	23	28	23	13

* Means of three replicates.



Phot. 2 Difference in fungitoxicity of Benlate (Ben.) and Bavistin (Bav.) towards sensitive (I) and resistant strains of *F. oxysporum* f. sp. *dianthi*. Note the differences in growth of sensitive strains at 1 ppm a.i. of the fungicides and also growth of the resistant strains at 1000-5000 ppm a.i. of both fungicides

In the comparison of the influence of other fungicides on highly resistant strains, the higher toxicity of benomyl than of MBC is striking. It is distinctly seen on Photo 2 and in Tables 3 and 4. The ED_{50} values for benomyl towards resistant strains lie within much lower concentrations limits than those for MBC (Table 6). This situation is opposite to that concerning strains sensitive to these fungicides. Methyl thiop-

Table 5

Growth of isolates of *Fusarium oxysporum* f. sp. *dianthi*, susceptible, tolerant and resistant to carbendazim (MBC) on potato dextrose agar (Difco); without fungicides; means of five replicates

Mean diameter of fungus colonies in mm after 7 days at 25°C									
Fungus strains									
sensitive		tolerant		resistant					
Fg-1	Fg-29	T-3	T-4	Fg-14	6.14	Fg-9	4.15	Fg-10	Fg-12
50a*	50a	50a	47a	34b	35b	26c	28c	12d	13d

$LSD_{P=0.01}=4.5$

* Means not followed by the same letter differ significantly at P=0.01.

Table 6

Fungistatic activity of fungicides towards mycelial growth of selected isolates of *Fusarium oxysporum* f. sp. *dianthi* (Prill et Dell.) Snyd. et Hansen

Isolates	ED_{50} (ppm a.i.)		
	benomyl	carbendazim (MBC)	thiophanate-methyl
1.14	0.6	0.4	1.3
1.17	0.5	0.3	1.2
1.19	0.5	0.3	1.3
Fg-3	0.6	0.4	1.5
Fg-23	0.4	0.3	1.2
Fg-21a	0.6	0.4	1.5
XVII	0.6	0.5	1.5
T-3	2.7	2.5-3.5	5.5
T-5	2.1	2.5	5.5
2.11	500	850	1500
4.13	500	1000-2000	3000
5.03	300	1000	2500
6.17	300-500	2000	2500
6.19	400-800	1500	2500
6.07	1000	2000-3000	5000

hanate, on the other hand is considerably less fungitoxic both towards resistant and sensitive strains (Tables 3, 4 and 6 and Phot. 2).

The growth habit of resistant colonies is somewhat different, they are denser and have a more profuse aerial mycelium (Phot. 1 and 2). Moreover, most resistant strains grow considerably slower both on medium without fungicide and in the range of fungicide concentrations which does not markerly influenced their growth. The differences can be seen on Photos 1 and 2 and in Tables 2, 3, 4 and 5. As regards growth, the resistant strains may be divided into two distinctly differing groups: (1) with very slow growth and (2) showing growth about

Table 7

Influence of carbendazim (MBC) on mycelial growth of tolerant and susceptible strains of *Fusarium oxysporum* f. sp. *dianthi* (Prill. et Del.) Snyd. et Hansen

Strains	Diameter of fungus colonies in mm after 9 days						
	Concentration of MBC (ppm)						
	0	0.3	0,5	1	3	5	10
Tolerant							
T-4	46	—	44	28	9	2	0
T-6	50	—	40	31	11	5	trace
Sensitive							
1.16	50	23	15	0	—	—	—
1.1	50	29	23	10	0	0	—

50-60 per cent slower as compared with the sensitive strains. Much rarer are resistant isolates which preserve a relatively rapid growth, comparable to that of sensitive strains. In most cases the isolates from one sample of diseased plants in one greenhouse were similar in their growth rate and reaction within the range of high MBC concentrations. On the other hand, wider differences were noted less frequently (in 3 greenhouses) between strains as regards the resistance level and growth of colonies.

DISCUSSION

Benzimidazole fungicides are used in Poland in the protection of greenhouse carnations more intensively than on other crops. It would seem, therefore, that no *Fusarium oxysporum* f. sp. *dianthi* population could avoid exposure to a longer or shorter action of these fungicides. It is very interesting however, that in some large greenhouses, where the soil is strongly infected with this fungus and large doses of Benlate have been applied, no resistant strains were found.

The high level of durable resistance to systemic fungicides may be ascribed, as in the case of other fungi, to mutational changes. Wide difference in resistance point to the complex character of the changes in the genetic material of the fungus. They probably arise by mutation involving several loci. This seems to indicate that benzimidazole fungicides must act not upon one, but on several links of the fungus metabolism. Although the mechanism of action of these agents is not yet well known, its connection with RNA metabolism (Bartels-Schooley and Mc Neil 1971; for review see Dekker 1972; Erwin 1973), effect on mitosis in fungi (Hastie 1970; Davidse 1973; Ben Yephet 1974) and the frequency of appearance of re-

sistance arouse some fears as regards the use of this group of fungicides.

To date, within *Fusarium oxysporum* high resistance to benzimidazole compounds in field conditions has been detected in forms of f. sp. *melonis* (Dassenoy and Mayer 1973), f. sp. *gladioli* (Magie and Wilfret 1974). In laboratory conditions very high and durable resistance has been found in *F. oxysporum* f. sp. *tulipae* (Valášková 1973) and reversible tolerance to relatively low concentrations of these fungicides in f. sp. *lycopersici* (Thanassoulopoulos et al., 1973) and in *F. solani* (Richardson 1973). A similar range of differentiation of resistance and tolerance of strains to benzimidazole fungicides was noted among some other fungi (Bollen and Fuchs 1970; Schmitthenner 1970; Hastie and Georgopoulos 1971; Ruppel 1975).

The resistance of *F. oxysporum* f. sp. *dianthi* to systemic fungicides was frequently associated with growth depression on unpoisoned media and also with changes in the appearance of the cultures. A similar situation was observed in other species (Wuest et al. 1974; Bollen and Scholten 1971; Georgopoulos et at. 1971). Part of the isolates grew three — others two-times slower than the sensitive strains, in some few growth depression was relatively slight, showing no noticeaple connection with the resistance level of these strains. All resistant isolates of *F. oxysporum* f. sp. *dianthi* grew distinctly slower, they formed a denser mycelium than the fast growing sensitive or low tolerant ones. In most cases homogenouos strains in this respect were isolated from single glasshouses, only from two farms were differing strains obtained.

By way of transfers in the presence of fungicides a higher tolerance to benzimidazole fungicides was achieved in some fungi (Thanassoulopoulos et al. 1971; Abelensiev 1973; Richardson 1973; Kataria and Grover 1974). In the case of *F. oxysporum* f. sp. *dianthi,* tolerant strains were isolated in production conditions. Among the resistant *F. Oxysporum* f. sp. *dianthi* isolates further selection for higher resistance was possible like in other cases (Valášková 1973; Bollen and Fuchs 1970; Ruppel 1975). Changes in tolerance were not observed in the case of one-spore subcultures. The tolerance of *Fusarium oxysporum* f. sp. *dianthi* to relatively low MBC concentrations was, as in other fungi (Bollen and Fuchs 1970; Kataria and Grover 1974) of reversible character and probably of the nature of enzymatic adaptation.

MBC was markedly more active than benomyl for sensitive strains, whereas Benlate was distinctly more active towards highly resistant ones. A similar phenomenon has been observed among benzimidazole

sensitive and resistant strains of *Ascochyta chrysanthemi* (Grouet 1974; Steekelenburg 1976).

Observations in production conditions indicate that resistant strains of *Fusarium oxysporum* f. sp. *dianthi* preserves a high pathogenicity, although the appearance of disease symptoms is slightly retarded. Experiments on the pathogenicity of chosen resistant strains are in progress.

Although a weaker efficiency of systemic fungicides has been remarked in the protection from carnation fusariosis (Tramier 1974; Rattink 1974), the fungus isolates proved, in these cases sensitive to the fungicides *in vitro* (full inhibifion at 1 ppm of benomyl). Slower development of disease symptoms on plants infected by resistant strains create additional hazard of spread the fungus with propagating plant material.

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Występowanie i charakterystyka form *Fusarium oxysporum* f. sp. dianthi (Prill et Del.) Snyder et Hansen odpornych na fungicydy systemiczne

Streszczenie

W ostatnich latach zaobserwowano wzrost zagrożenia goździków szklarniowych w Polsce przez *Fusarium oxysporum* f. sp. *dianthi* (Prill et Del.) Snyd. et Hans. Wśród izolatów grzyba pochodzących z wielu szklarni stwierdzono częste występowanie szczepów wysoce odpornych na fungicydy systemiczne z grupy benzimidazoli (Benlate, Bavistin, Funaben) i metylotiofanat (Topsin M). Wiele z nich nie reagowało na obecność w pożywce 1000 ppm MBC i rosły jeszcze przy 10 000 ppm. Odporność ta jest trwała. Większość szczepów odpornych, rośnie 2-3-krotnie wolniej od szczepów wrażliwych, a nieliczne tylko o 20-30% wolniej. Wśród szczepów odpornych występuje zróżnicowanie względem granicznych stężeń tolerowanych.

Zauważono pewne zróżnicowanie względem MBC wśród szczepów wrażliwych (ED_{50} MBC = 0,3-0,4 — czasem 0,5 ppm). W 2-ch przypadkach izolowano szczepy o niskiej tolerancji (ED_{50} MBC ok. 2,0-2,5 ppm). Drogą pasaży na pożywkach z MBC uzyskano w 4 przypadkach adaptacyjną tolerancję (ED_{50} = 2 ppm MBC). Tolerancje te mają tendencję do zanikania na pożywkach bez fungicydu.

Wśród izolatów odpornych na 500 ppm MBC drogą pasaży można było zwiększyć odporność kilkakrotnie. Nie uzyskano natomiast zmian w reakcji na MBC przez pasażowanie szczepów jednozarodnikowych.

MBC był bardziej aktywny względem szczepów wrażliwych (ED_{50} = 0,3-0,4 ppm) niż benomyl (ED_{50} = 0,4-0,6 ppm), natomiast benomyl był zdecydowanie bardziej aktywny względem szczepów odpornych (pełna inhibicja wielu szczepów przy 1000 ppm) niż MBC (wzrost wielu szczepów jeszcze przy 5-10 tys. ppm).

Tego typu odporność *F. oxysporum* f. sp. *dianthi* na fungicydy systemiczne nie była dotychczas notowana w literaturze.