FUNGICIDAL EFFECTS OF SOME CHEMICALS ON *SCLEROTINIA TRIFOLIORUM* ERIKSS.

AARRE YLIMÄKI

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Department of Plant Pathology, Agricultural Research Centre, Tikkurila, Finland

In field trials carried out by the Department of Plant Pathology it has been established that clover rot (*Sclerotinia trifoliorum* Erikss.) can effectively be controlled by PCNB (quintozene-)-preparations (YLIMÄKI 1955, 1956, 1969). The effect of these fungicides and their dependence on environmental factors as well as the effectiveness of some other chemicals on *S. trifoliorum* has been studied.

Methods

Since the effect of fungicides on the growth of mycelium in *S. trifoliorum* is easier to study, and as fumigation of fungicides appears to be a relatively advantageous, the studies were carried out as plate tests. The chemicals were placed in weighed amounts on four filter papers of 10 mm. These were placed on the surface of agar from where at least the watersoluble chemicals could diffuse along the agar surface. The fungus was transferred into the middle of the plate (c.f. Fig. 1). In some cases the fungicides were dusted or sprayed direct on the mycelium which had already started to grow.

To establish merely the fumigation effect of the chemicals, the substance under study was placed in a small plastic cup inside the cover of the Petri dish, upside down and with the fungus on the medium above it.

The growth of the mycelia was measured daily and observations were made on the formation of sclerotia. Microscopical studies were made to see whether the chemicals had caused visible changes in the mycelia. From the treated dishes the mycelia and sclerotia were transferred again on the normal medium so as to established whether the treatment had caused permanent changes in the mycelia. For a study of the sporophores the sclerotia were germinated on water agar or on wet quartz sand.

Results

The experiments (Table 1, Figs. 1—4) showed that the chemicals had a relatively restraining effect on the mycelium of *S. trifoliorum*, but as many of these substances are

Table 1. Effect of various chemicals on *Sclerotinia trifoliorum* in laboratory trials.

Preparation	Active ingredients	Mycelial growth mm after days		
		2	6	12
	Mercuric compounds			
Agrosan GN	phenylmercury acetate 0.85 % + ethylmercury chloride 0.15 %	9.5	28.4	33.7
Atiran	methoxyethylmercury chloride 4.6 %	16.3	29.9	44.9
Ceresan Nb.	methoxymercury acetate 2.4 %	16.3	26.3	31.4
CRC	mercurochloride 4 %	9.5	59.0	100
Duphar mercury spray	phenylmercury cellulose ether 4.3 %	11.1	29.5	35.3
Femma	phenylmercury acetate 2.0 %	10.5	43.2	51.6
Germisan	phenylmercury pyrocatechine 3.3 %	9.5	33.7	40.0
Mercadmine	phenylmercury salicylate 5 %	4.8	23.2	25.3
Solusan	methylethylmercury acetate (Hg 15,0 %)	7.4	18.9	20.0
Täyssato	methoxyethylmercury chloride 2.2 %	7.9	29.5	66.8
Verdasan	phenylmercury acetate 5 %	11.6	29.3	31.0
YF 5049	phenylmercury salicylate anilide 96 g/l	5.3	15.8	15.8
	Average	10.0	30.6	41.3
	Benzenes			
Amatin Staub	hexachlorobenzene (HCB) 20 %	12.1	61.1	97.4
Avicol dust	quintozene (PCNB) 20 %	0	9.4	31.8
Avicol wp.	„ 50 „	4.8	13.2	26.1
Botrilex	„ 20 „	4.6	20.3	53.1
Brassicol dust	„ 20 „	7.5	18.9	44.5
Brassicol sup.	„ 50 „	6.0	20.2	38.8
Fartox dust	„ 20 „	9.4	22.4	24.1
Folosan	tecnazene (TCNB) 5 „	1.2	5.1	11.7
Olpisan	trichlorodinitrobenzene (TCDNB) 20 %	4.2	15.4	39.6
Bulbosan	trichlorotrinitrobenzene (TCTNB) 7.5 %	5.3	12.6	26.8
Bulbosit	rodandinitrobenzene (RDNB)	10.0	47.4	73.7
	Average	5.2	40.1	38.0
	Thiocarbamates			
Dithane Z-78	zineb 65 %	12.6	63.2	92.1
Duphar ferbam	ferbam 95 %	13.7	38.2	52.1
	Average	13.2	50.7	72.1
	Captan compounds			
Orthocide 75	captan 75 %	16.3	63.7	89.0
Orthocide 50	captan 50 %	0	57.0	76.3
	Average	8.2	60.4	82.7
	Copper compounds			
Kuprijauhe	copper oxychloride 85 %	18.9	87.5	100
KT 35	„ 60 %	27.1	70.6	70.6
	Average	23.0	79.1	85.3

Preparation	Active ingredients	Mycelial growth mm after days 2	6	12
	Antibiotics			
Actidione	cycloheximide 85—100 %	15.3	22.0	22.8
Agrimycin	streptomycin 15 % + oxytetracycline 1.5 %	27.1	100	100
Griseofulvin	griseofulvin 98 %	22.6	76.3	88.4
Kojic acid	5-hydroxy-2 hydroxine-ethyl-4 pyrone	30.7	100	100
Sorbistat	sorbic acid	26.7	70.8	84.7
Usno	usnic acid	29.2	98.8	96.5
U-4527	cycloheximide	19.4	25.9	26.8
U-7413	„ -oxime	25.2	75.9	81.6
U-7414	„ -acetate	27.6	97.5	98.7
U-7415	„ -semicarbazone	25.7	85.1	86.8
	Average	25.0	75.2	78.6
	Other compounds			
Pomarsol forte	thiram 80 %	12.4	40.8	59.2
Fusarex plus	tecnazene 2 % + isopropyl N-phenylcarbamate (IPC) 1 %	0	11.6	21.1
KT 6	quintozene (PCNB) 5 % + isopropyl N-phenyl-carbamate (IPC) 1 %	7.4	35.3	52.5
VP 19—40 = Brestan	triphenyl stannic acetate 20 %	9.2	36.1	56.3
Bayer 4934	urbasulf 32 % (As 20 %)	11.2	21.8	23.5
Riedel B/500	oxychinolin halogen deriv. 1.0 %	2.7	44.8	100
Tuset	thiram 40 % + zineb 20 %	11.6	31.6	91.1
Spergon	chloranil 96 %	0	41.1	72.6
Phygon	dichlone 50 %	0	14.7	57.9
Belvitan K	methyl α-naphtylmethylether (MNME) 4 %	—	—	—
Controls		14.3	68.8	92.6

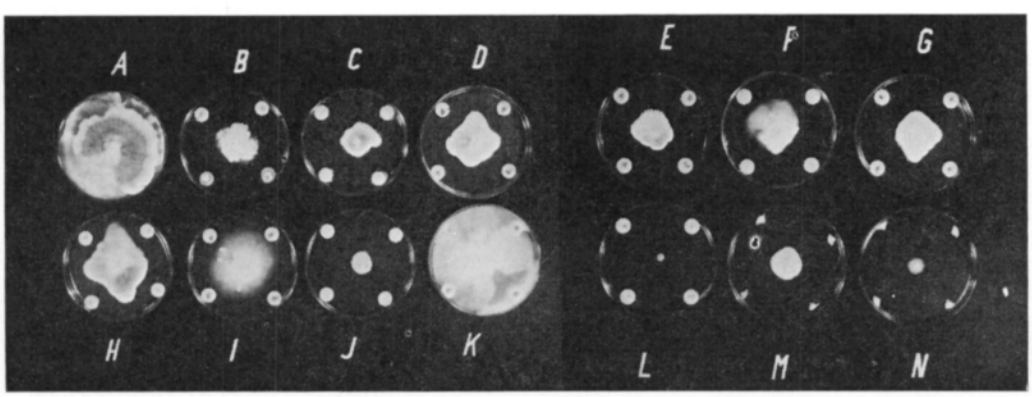

Fig. 1. Effect of various fungicides on the growth of *Sclerotinia trifoliorum* mycelium. A. control, B. Brassicol super wp., C. Verdasan, D. Germisan, E. Täyssato, F. Solusan, G. CRC, H. Agrosan GN, I. Atiran, J. Duphar mercury spray, K. Femma, L. Granosan, M. Mercadmine, N. YF 5049.

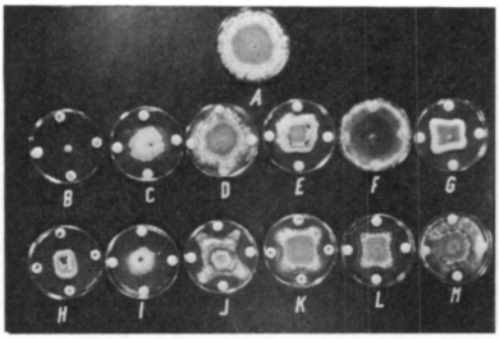

Fig. 2. Effect of various fungicides on the growth of *Sclerotinia trifoliorum* mycelium. A. control, B. Folosan, C. Brassicol, D. Dithane Z-78, E. Atiran, F. Kuprijauhe, G. Verdasan, H. Ceresan Nb., I. Brassicol wp., J. Brestan, K. Duphar ferbam, L. Pomarsol forte, M. Orthocide 75.

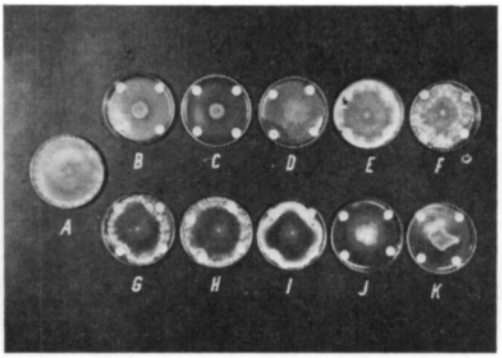

Fig. 3. Effect of various antibiotics on the growth of *Sclerotinia trifoliorum* mycelium. A. control, B. Actidione, C. U-4527, D. U-7413, E. U-7414, F. U-7415, G. Griseofulvin, H. Usno, I. Sorbistat, J. Brassicol wp., K. Verdasan.

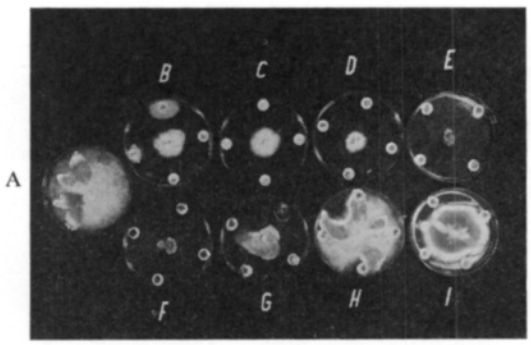

Fig. 4. Effect of various benzene-preparations on the growth of *S. trifoliorum* mycelium. A. control, B. Brassicol wp. C. Olpisan, D. Bulbosan, E. Folosan, F. Fusarex plus, G. KT 6, H. Amatin Staub, I. Bulbosit.

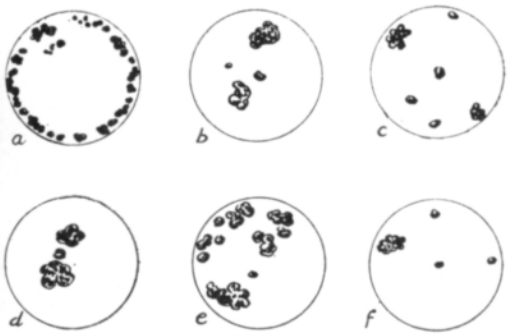

Fig. 5. Effect of fungicides on the formation of sclerotia in petri dishes. a. control, b. MNME, c. PCNB, d. zineb, e. captan, f. TCNB.

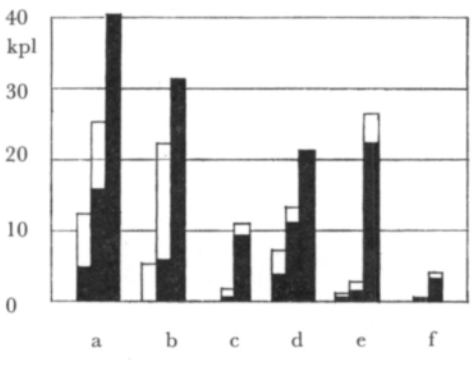

Fig. 6. The same trial as in Fig. 5. Number of sclerotia 10, 20, 39 days after inoculation. □ light sclerotia ■ black sclerotia

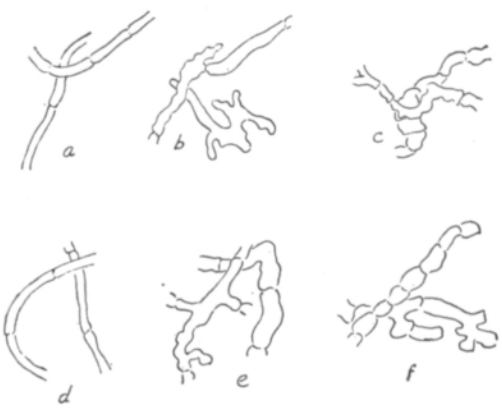

Fig. 7. Effect of some fungicides on the growth of mycelia of *S. trifoliorum*. a. control, b. MNME, c. PCNB, d. zineb, e. captan, f. TCNB.

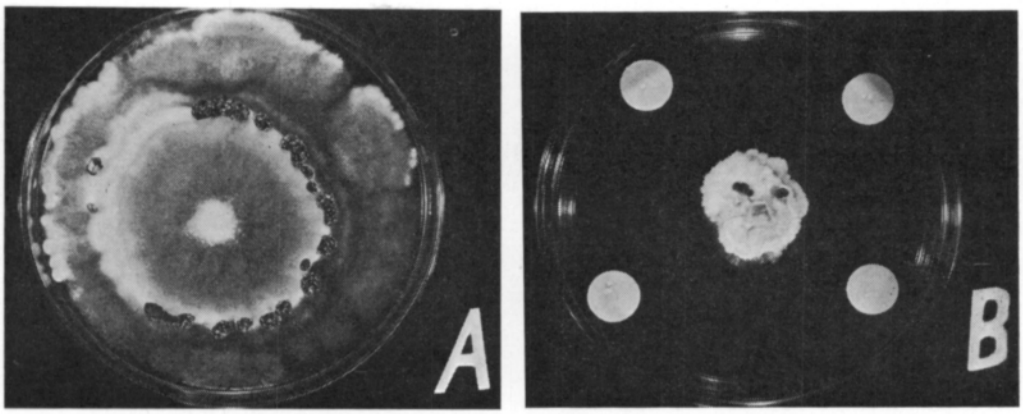

Fig. 8. Effect of PCNB preparations on the growth of mycelia and formation of sclerotia of *S. trifoliorum*. A. control, B. Avicol wp.

Table 2. The preservation of the efficacy of TCNB and PCNB preparations in open dishes.

Preparation		Duration of trial	Growth of mycelium mm	rel.
	A. At room temperature			
Folosan	TCNB	10 days	9.0	10
Brassicol	PCNB	,,	36.3	38
Control		,,	95.0	100
L.S.D.			4.7***	
Folosan	TCNB	10 months	45.8	48
Brassicol	PCNB	,,	86.3	90
Control		,,	95.0	100
L.S.D.			14.4***	
	B. Out of doors			
Folosan	TCNB	16 days	15.2	17
Avicol	PCNB	,,	76.4	85
Control		,,	90.0	100
L.S.D.			6.8***	

poisonous it is not possible to use them on grasslands. Moreover, the substances whi chin laboratory trials were rather effective, have proved to be less effective in field trials than the PCNB substances, which in field trials have effectively controlled the damage of clover rot (YLIMÄKI 1969).

When TCNB, PCNB, or captan preparations, were sprinkled on the mycelia transferred to the medium the growth of the mycelia of *S. trifoliorum* and the formation of sclerotia were seriously disturbed. TCNB was more effective in preventing the growth of mycelia than PCNB (Fig. 4), whereas the preventive effect on the formation of sclerotia was very similar in both (Figs. 5 and 6).

Table 3. Preservation of TCNB and PCNB preparations in different conditions

Way of storage	Preparation		Growth of mycelium mm	rel.
Trial I				
In a room, closed dish	Brassicol sup.	PCNB	32.5	34
,,	Brassicol dust	,,	44.5	47
,,	Avicol wp.	,,	23.5	25
,,	Folosan	TCNB	7.0	7
In a room, open dish, dry	Brassicol sup.	PCNB	39.0	41
,,	Brassicol dust	,,	43.5	46
,,	Avicol wp.	,,	25.5	27
,,	Folosan	TCNB	8.5	9
In a room, open dish, wet	Brassicol sup.	PCNB	31.5	33
,,	Avicol wp.	,,	44.5	47
Out of doors, open dish, wet	Brassicol sup.	PCNB	45.0	47
,,	Brassicol dust	,,	51.0	54
,,	Avicol wp.	,,	26.5	28
,,	Folosan	TCNB	12.5	13
Control			95.0	100
L.S.D.			4.4***	
Trial II				
In a room, closed dish	Folosan, an old amount	TCNB	12.5	14
,,	Folosan, an new amount	,,	18.0	20
,,	Botrilex, an old amount	PCNB	57.5	63
,,	Botrilex, an new amount	,,	69.3	76
In a room, open dish	Folosan	TCNB	41.0	45
,,	Botrilex	PCNB	79.5	88
In a cool place, open dish	Folosan	TCNB	19.8	22
,,	Botrilex	PCNB	32.3	36
Control			90.7	100
L.S.D.			12.6***	

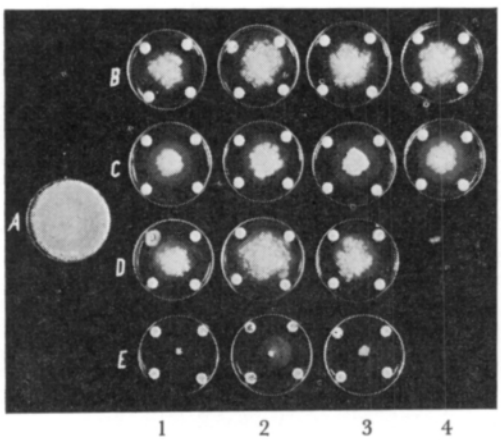

Fig. 9. Evaporation trial with benzene preparations. A. control, B. Brassicol wp., C. Avicol wp., D. Brassicol dust, E. Folosan; storage 1. in closed parcel 2. in open dish out of doors, wet 3. in open dish in a room, dry 4. open dish in a room, wet.

Table 4. Effect of temperature on the preservation of PCNB preparations. Substances preserved in open dishes during one month.

Preparation	Growth of mycelium Temperature (15—37°C) mm	rel.	(4—18°C) mm	rel.
Brassicol super wp.	31.0	41	24.0	32
Avicol wp.	23.5	31	27.0	36
Avicol dust	24.0	32	24.0	32
Botrilex dust	26.5	35	25.0	33
Brassicol dust	25.0	33	25.0	33
Control	75.0	100	—	—
L.S.D.			9.0***	

Observed microscopically, the mycelia which had interrupted their growth, seemed alive, even if abnormally thick and crooked (Fig. 7). The formation of appressors was more abundant in dishes treated with fungicides than in untreated dishes. The sclerotia formed more rapidly in control dishes. They were mainly on the edges of the dishes, whereas in the dishes treated with chemicals their occurrence was haphazard and they appeared mostly in groups joined to each other (Fig. 5). When transferred to an untreated medium, the abnormal mycelia and sclerotia were able again to form mycelia and sclerotia that seemed to be totally healthy.

The sclerotia from the mycelia treated with TCNB as well as with PCNB preparations formed numerous sporophores, they developed no apothecia, however, whereas in untreated sclerotia they developed normally.

Although pieces taken from the mycelia treated with both substances and placed on nonpoisonous medium formed normal kind of mycelium and sclerotia, these sclerotia did not develop normal sporophores as did the sclerotia of the control. It seems possible that TCNB and PCNB substances may have effects of more lasting nature on the *S. trifoliorum* fungus.

Preventing the growth of the mycelia of *S. trifoliorum* and the formation of sclerotia is clearly a fungistatic process (cf. Strecker 1957). The activity of these substances is dependent on the temperature: in the same temperature TCNB is more fungistatic than PCNB. Since the vapor pressure of the TCNB is 4—5 times greater in the same temperature as the vapor pressure of PCNB (Reavill 1954), the difference in the efficacy of the substances may depend mainly on the difference in fumigation. In all trials where the TCNB preparation was used, it was more effective on *S. trifoliorum* than the PCNB preparations (Tables 2—3, Figs. 2, 4 and 9).

As the preventive treatments in the control of clover rot should be started rather early (Ylimäki 1969), it is important to know how long the fungicide remains effective in field conditions. The length of the time the substances preserve their effectiveness in storage should also be known. To study these qualities, the TCNB and PCNB preparations were kept in different temperature and humidity conditions.

In the trials the effect of the temperature, the humidity and the air current on the effectiveness of TCNB and PCNB was so small that the above factors cannot have a

perceptibly lowering effect on the usability of TCNB or PCNB on the fields in autumn conditions (Table 4). In fact, the substances may loose their effectiveness when stored at higher temperatures in open or badly closed covers. In the trials this applied in particular to the PCNB preparations (Tables 2 and 3).

Summary

In efficiency tests carried out in laboratory (Table 1, Figs. 1—4) it was established that on media many chemicals had a restraining effect on the growth of the mycelia of *Sclerotinia trifoliorum*. In addition to PCNB preparations, TCNB substances very severely restrained the growth of mycelia and the formation of sclerotia of the fungus (Figs. 5—8). Sclerotia formed of mycelia treated with TCNB as well as with PCNB substances developed numerous sporophores which were not fertile however. The effect of both PCNB and TCNB on the mycelium of *S. trifoliorum* proved only restricting on the growth. It did not destroy the mycelia. The activity of the substances is dependent on the temperature and the higher effectivity of TCNB is based on its greater degree of vaporization (Tables 2—3, Figs. 2,4 and 9). In autumn and in outdoor conditions on the field the vaporization of the substances is so small that it does not lower the effectiveness to any great extent (Tables 2—3, Fig. 9).

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SELOSTUS

ERÄIDEN KEMIKAALIEN VAIKUTUS *SCLEROTINIA TRIFOLIORUM* SIENEEN

Aarre Ylimäki

Kasvitautien tutkimuslaitos, Maatalouden tutkimuskeskus, Tikkurila

Laboratoriossa suoritetuissa tehokokeissa (taulukko 1, kuvat 1—4) ilmeni, että monilla kemikaaleilla oli ravintoalustoilla *S. trifoliorum*'in rihmaston kasvua ehkäisevä vaikutus, mutta useat niistä eivät kuitenkaan ole käyttökelpoisia nurmilla myrkyllisyytensä takia. PCNB-valmisteiden ohella myös TCNB-aineella oli erittäin voimakas sienen rihmaston kasvua ja rihmastopahkojen muodostumista ehkäisevä vaikutus (kuvat 5—8). Sekä TCNB- että PCNB- aineilla käsitellyistä rihmastoista muodostuneet rihmastopahkat kehittivät lukuisia itiöemien aiheita, jotka kuitenkaan eivät olleet fertiilejä.

Sekä PCNB:n että TCNB:n vaikutus *S. trifoliorum*'in rihmastoon oli vain kasvua ehkäisevä, ei tappava. Aineiden aktiivisuus on riippuvainen lämpötilasta ja perustuu TCNB:n suurempi tehokkuus sen suurempaan kaasuuntuvuuteen (taulukot 2—5, kuvat 2, 4, 9). Syksyisin ulkona pellolla vallitsevissa sääoloissa on aineiden kaasuuntuvuus kuitenkin siksi vähäinen, ettei sillä ole mainittavasti tehoa heikentävää vaikutusta (taulukot 2—5, kuva 9).