EVALUATION OF FUNGICIDES FOR CONTROL OF TREE DISEASES

II. Screening Against the Dutch Elm Disease *Ceratocystis ulmi* (Buism) C. Moreau Under Laboratory Conditions.

by

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RÉSUMÉ

On a fait le tri de 20 composés différant par la structure et les propriétés chimiques, pour leur action contre la maladie hollandaise de l'orme. Cela s'est passé en laboratoire, sur milieu de culture patate-dextrose-agar. On a évalué la puissance relative de ces composés au moyen de ED_{50}; c'est ainsi que le Lignasan® s'est révélé le plus toxique de tous, suivi de l'Acti-Dione, du Baydam-18654, du Bénomyl® et du Dowicide-A. On étudie plus en détail les propriétés endothérapiques du Baydam-18654 et du Dowicide-A.

INTRODUCTION

Elm (Ulmus americana L.) is one of the most popular shade trees in urban and suburban localities in North America and its protection against the Dutch elm disease (DED) caused by Ceratocystis ulmi (Buism) Moreau, is urgently needed. Even though considerable effort had been made during the past 40 years to save the elm from DED, no effective fungicide was available until the discovery of benomyl® by the DuPont Co., U.S.A. (Delp and Klopping 1968). It seems that DED can now be contained and combatted (Smalley 1972, Prasad 1972). Although benomyl shows great promise as a chemotherapeutant against the DED (Stipes 1969, Biehn and Diamond 1971) its low solubility in aqueous formulations is a serious drawback. Also, if strains of C. ulmi became resistant to benomyl as has been the case with other pathogens, (Berger 1973; Schroederer and Provvidenti 1969, Litterel 1974; Clark et al 1974) it would be wise to anticipate this and screen other compounds which possess greater solubility, compatibility with spray formulations, and potency against the DED. With this objective in mind, a screening program was first initiated in 1972 (Prasad and Travnick 1972) and the present report describes the relative potencies of twenty newer compounds of varied chemical and physical structures against the DED with the aid of an improved bioassay technique.

MATERIAL AND METHODS

(i) Chemical Compounds

Samples of twenty commercially available compounds, currently available on the market were used in these tests. The details of their

chemical structures and description together with the name of chemical companies supplying them are given in the appendix.

A 100% activity factor for each fungicide was calculated on the basis of the active ingredient and then appropriate amounts were weighed to prepare solutions or suspensions. Further dilutions were made to obtain a range of concentrations for each candidate compound under study. Four concentrations of each sample together with an appropriate control were employed for screening against the DED. In order to prevent undue decomposition, solutions were stored in a cool and dark place.

(ii) Culture of Fungi

Pure cultures of vigorously growing strains of Ceratocystis ulmi (Buism) Moreau, were obtained from the Great Lakes Forest Research Centre, Sault Ste. Marie, Ontario, and their pathogenicity was tested on young trees. Then the reisolates were subcultured and grown in standard Petri dishes (100 x 15 mm) containing 15 ml. of potato-dextrose-agar (PDA). When the colony formation was excellent in these plates, disks of 10 mm. diameter were cut out with a #7 cork borer and transferred to another set of Petri dishes plated with 15 ml. PDA and the appropriate concentration of the candidate fungicide. All these operations were performed under aseptic conditions.

(iii) Fungicide Treatment

The screening of each candidate fungicide was carried out at five concentrations: 10 ppm; 100 ppm; 1000 ppm, and 5000 ppm. A sterile PDA medium was prepared in an autoclave and when the agar had cooled down to about 100° F, a sufficient amount of fungicide was added to 30 ml. of agar in a beaker and divided equally between 2 petri dishes, thus each containing 15 ml of media and the appropriate concentration of the fungicide.

The plates were then stored in a refrigerator for future use. Subsequently the screening was done by placing three disks of pure Ceratocystis ulmi culture on each of two plates containing a mixture of PDA and the candidate compound. In this way six replicates of each concentration were used to measure the growth response of the fungus. Measurements of zones of inhibitions were taken at 2, 4, and 6 day intervals and the growth in each plate was compared to that of the controls, (Fig. 6). From a series of growth curves, the effective concentration halving the rate of colony formation (ED_{50}) for a 6 day period was computed and this parameter (zone of inhibition) was employed to compare relative potency of each candidate compound.

RESULTS AND DISCUSSION

The dose-response curves for each compound are presented in Figs. 1, 2, 3, 4 and 5. It is evident that some candidate chemicals are more potent than others. It appears that time of exposure to the fungicide is a factor in toxicity since treatment for 2, 4 and 6 days produced different degrees of responses. Of interest is the stimulatory effects of low concentration of some fungicides. (Daconil and Cupramar, Figs. 2 and 3). Since the dose response data could not be analysed by the standard probit technique, ED_{50} values were estimated by approximation and the results are given in Table 1. For better comparison, relative inhibition and inhibition index were transferred from these data according to the procedure outlined by Prasad and Travnick (1972). It seems that the present method of mixing the fungicide directly with the agar and then implantation of DED disks, is superior to the previously used "well technique" (Prasad and Travnick 1972) because the fungicide had a chance to

uniformly distribute and diffuse throughout the agar medium. From Fig. 6 it can be seen that the pattern of colony formation was quite consistent and therefore this method seemed very suitable for screening of fungicides with lower solubility limits. Whether some decomposition took place during mixing with warm agar (40^{o} C) remains to be ascertained but according to physico-chemical data supplied by manufacturers, many of the compounds tested herein had much higher melting points than 40^{o} C, and therefore it was unlikely that slightly warm agar caused undue degradation of the candidate compounds.

Judging the relative potency on the basis of ED_{50} it seems clear that Lignasan is the most fungitoxic compound followed by Actidione, Baydam-18654, benomyl and MBC-Cl; the latter two produced equal effects. Because higher concentrations were needed to provide the same degree of response (ED_{50}) by Dowicide A, Du-ter, Calixin, Zyban and others, these compounds must be less potent than the above group. Similarly there were other compounds like Cupramar, Daconil, Captan and Agro-Chem that were least effective and their use as fungitoxicants must be questioned. R-28921, Validacin, PP 395, meltatox, Plonderel and Vitavax can be regarded as potential fungicides for DED control but their usage must await further testing for systemicity. Often many compounds are found to be extremely fungitoxic at low concentration in the agar plate, but they fail to translocate in the host trees or they produce phytotoxic effects and as a consequence cannot be effectively employed as systemic material for the control of vascular diseases in forest trees. Therefore, the next logical step would be to investigate penetration and translocation patterns of some of the promising candidates using potted seedlings in the greenhouse. Only then further selection should be made from this list of twenty compounds.

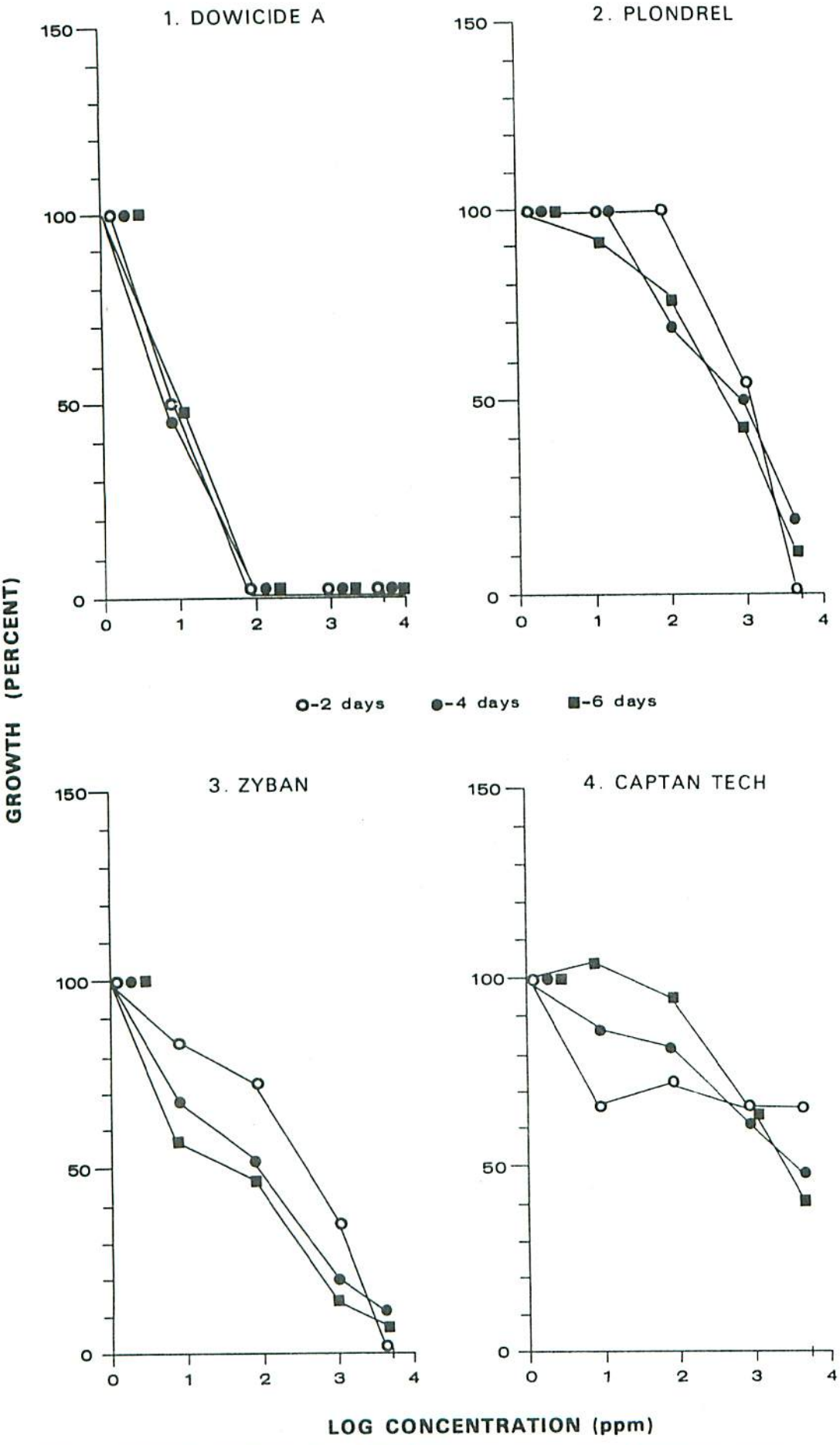

Fig. 1 Dose-response curves of Ceratocystis ulmi to treatment with Dowicide A, Plondrel, Zyban, and Captan Tech for 2, 4 and 6 days.

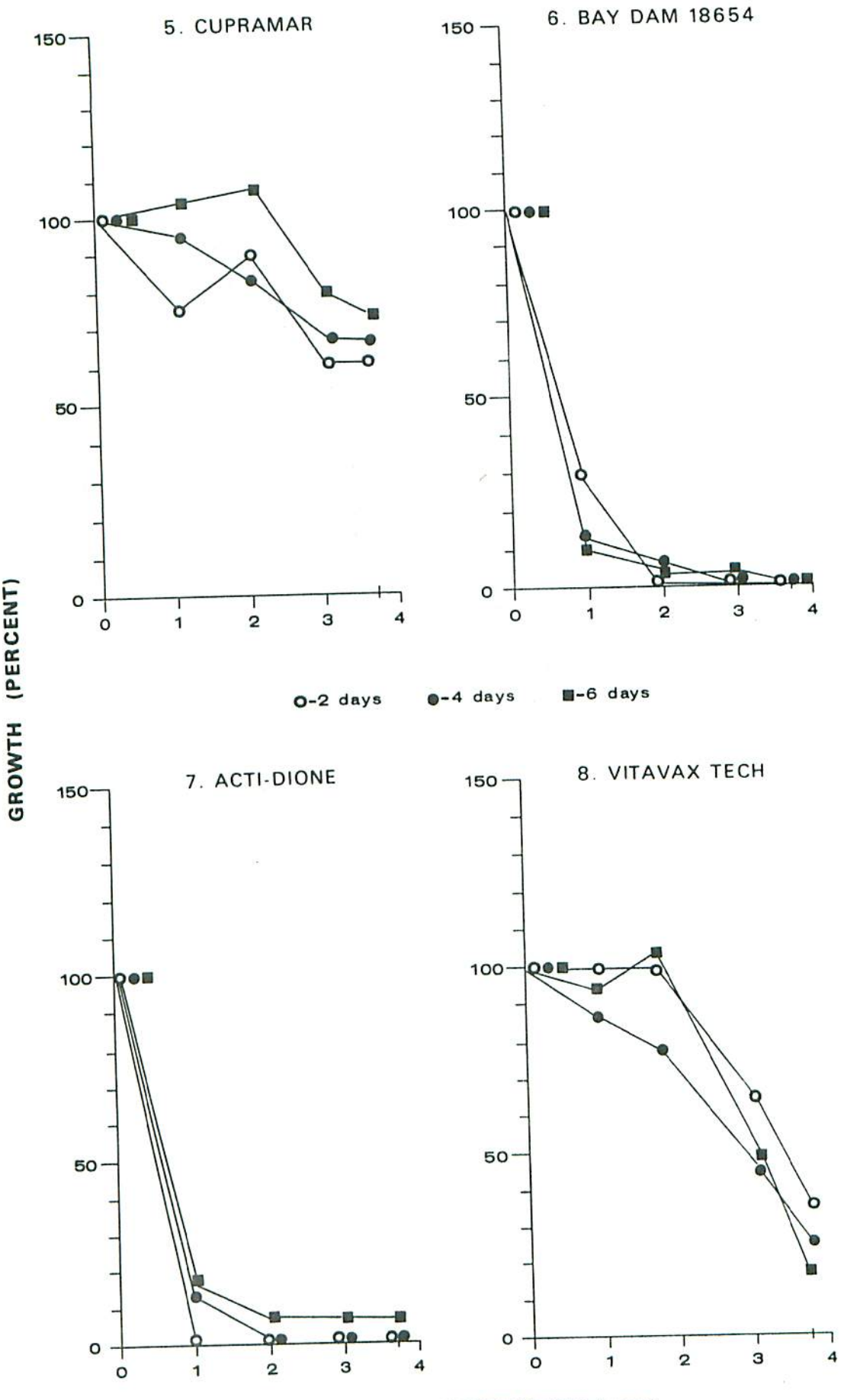

Fig. 2 Dose-response curves of Ceratocystis ulmi to treatment with Cupramar, Bay Dam 18654, Acti-Dione, and Vitavax Tech for 2, 4, and 6 days.

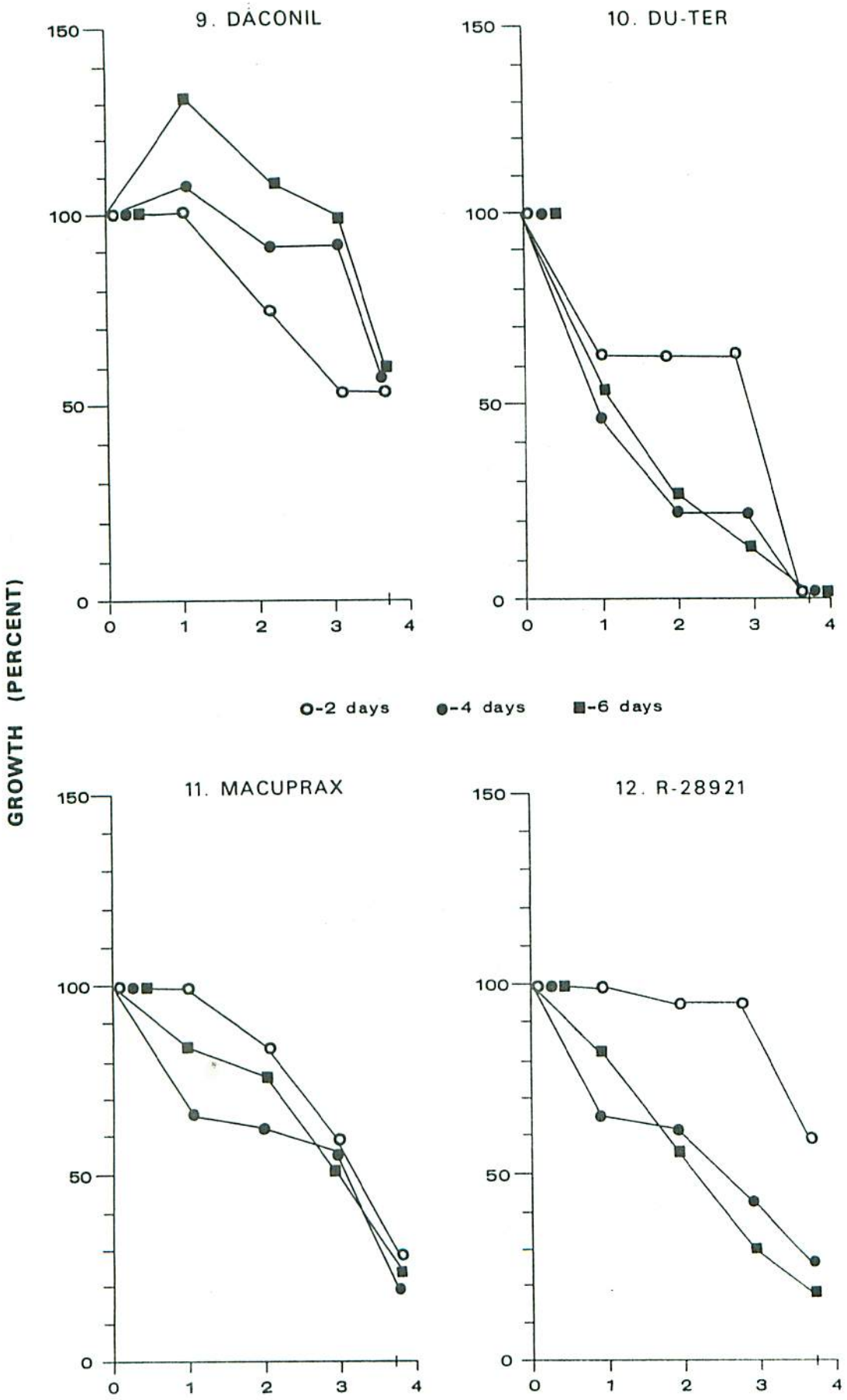

Fig. 3 Dose-response curves of Ceratocystis ulmi to treatment with Daconil, Du-Ter, Macuprax and R-28921 for 2, 4 and 6 days.

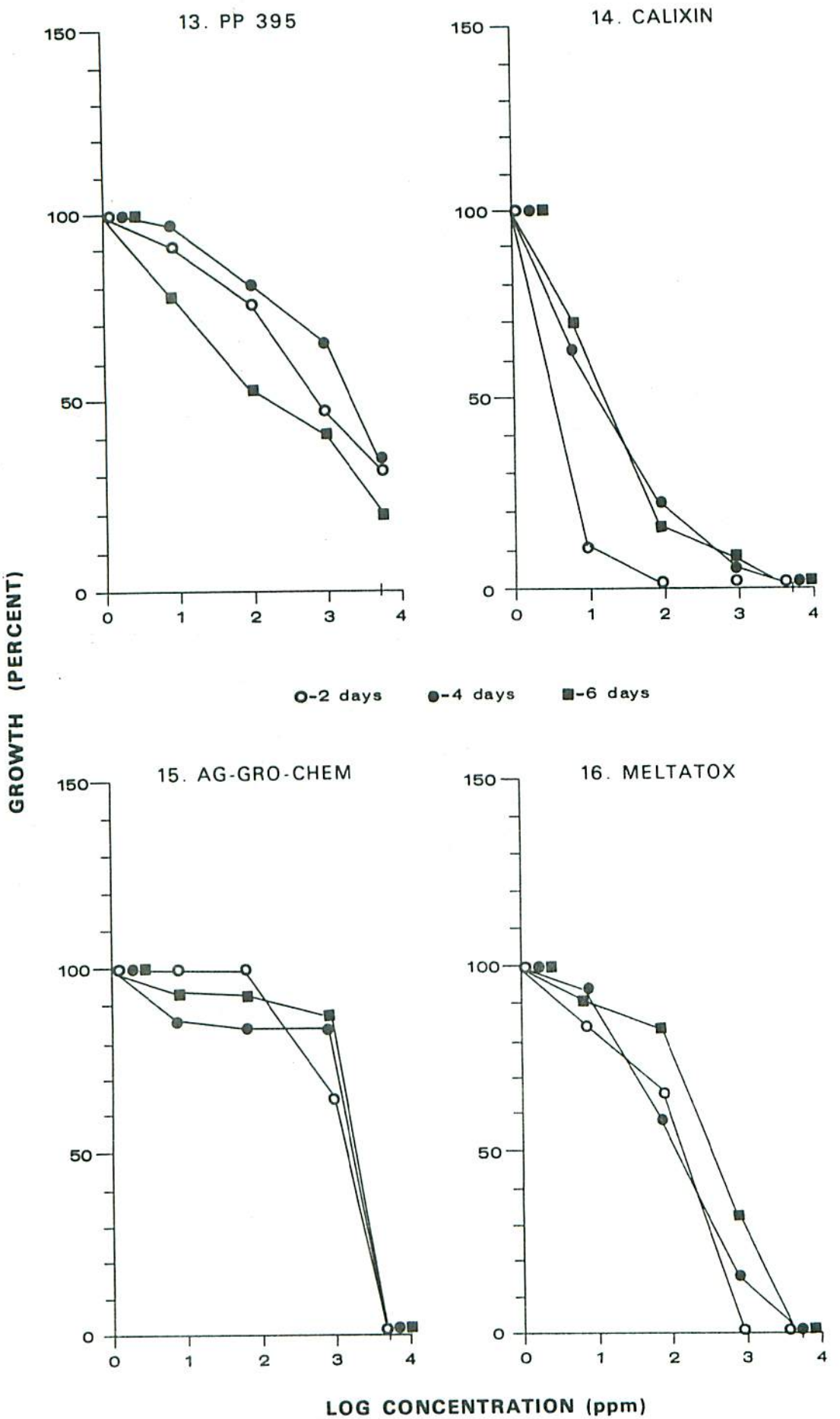

Fig. 4. Dose-response curves of Ceratocystis ulmi to treatment with PP 395, Calixin, AG-GRO-CHEM and Meltatox for 2, 4 and 6 days.

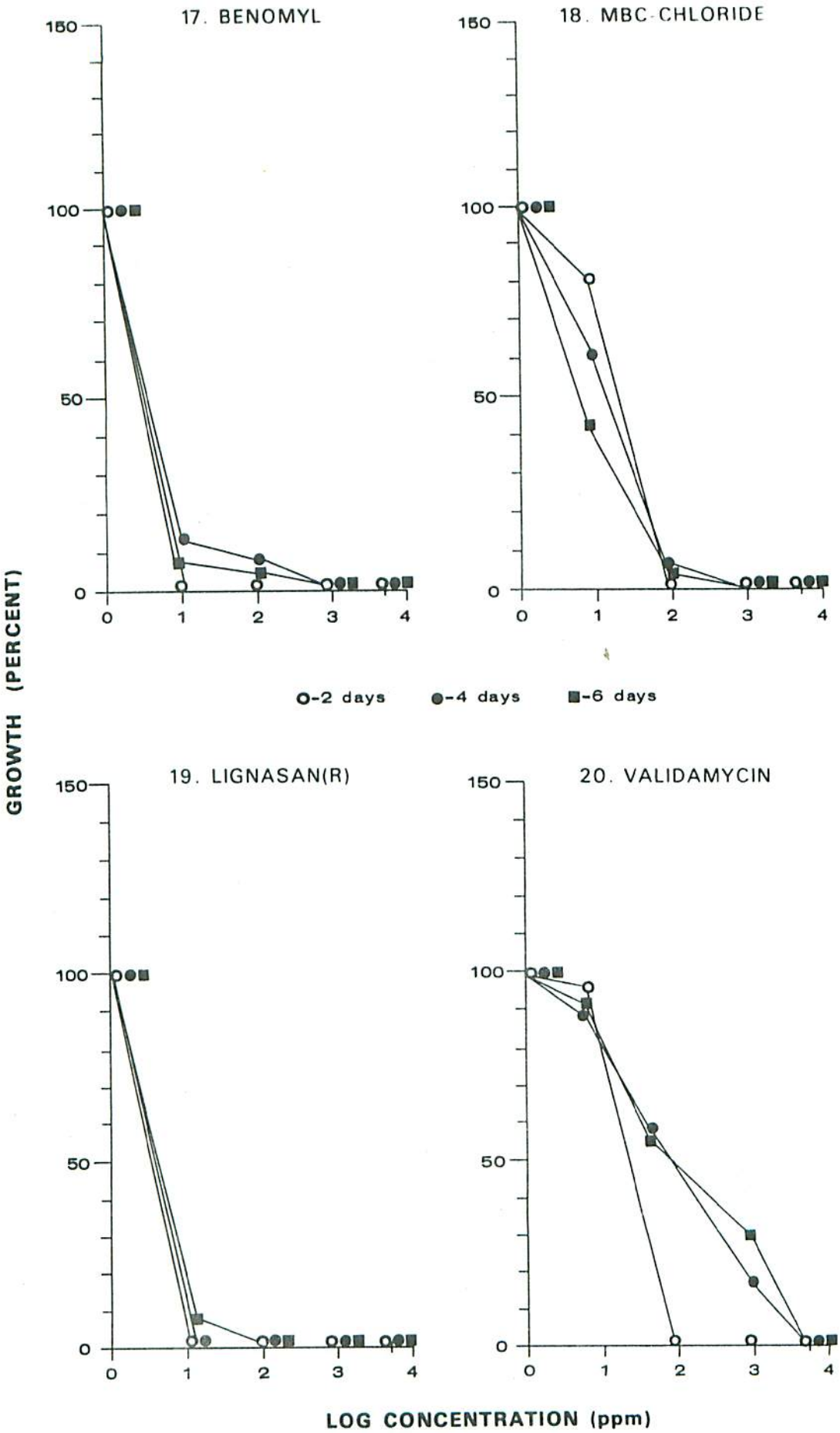

Fig. 5 Dose-response curves of Ceratocystis ulmi to treatment with Benomyl, MBC-chloride, Lignasan and Validamycin for 2,4 and 6 days.

TABLE I

COMPARATIVE TOXICITY OF COMPOUNDS TESTED AGAINST CERATOCYSTIS ULMI AFTER SIX DAYS

FUNGICIDE	ED_{50} (ppm)	RELATIVE INHIBITION	INHIBITION INDEX
1. LIGNASAN	3	1.66	166
2. ACTI-DIONE	5	1.00	100
3. BAY-DAM 18654	5	1.00	100
4. BENOMYL	5	1.00	100
5. MBC-HC1	5	1.00	100
6. DOWICIDE A	10	0.50	50
7. DU-TER	20	0.25	25
8. CALIXIN	30	0.17	17
9. ZYBAN	40	0.13	13
10. R-28921	200	0.03	3
11. VALIDACIN	200	0.03	3
12. PP 396	250	0.02	2
13. MELTATOX	500	0.01	1
14. PLONDREL	500	0.01	1
15. VITAVAX	1000	0.005	0.5
16. MACUPRAX	1500	0.003	0.3
17. AG-GRO-CHEM	2000	0.002	0.2
18. CAPTAN	2200	0.002	0.2
19. DACONIL	5000	0.001	0.1
20. CUPRAMAR	100000	0.0	0.0

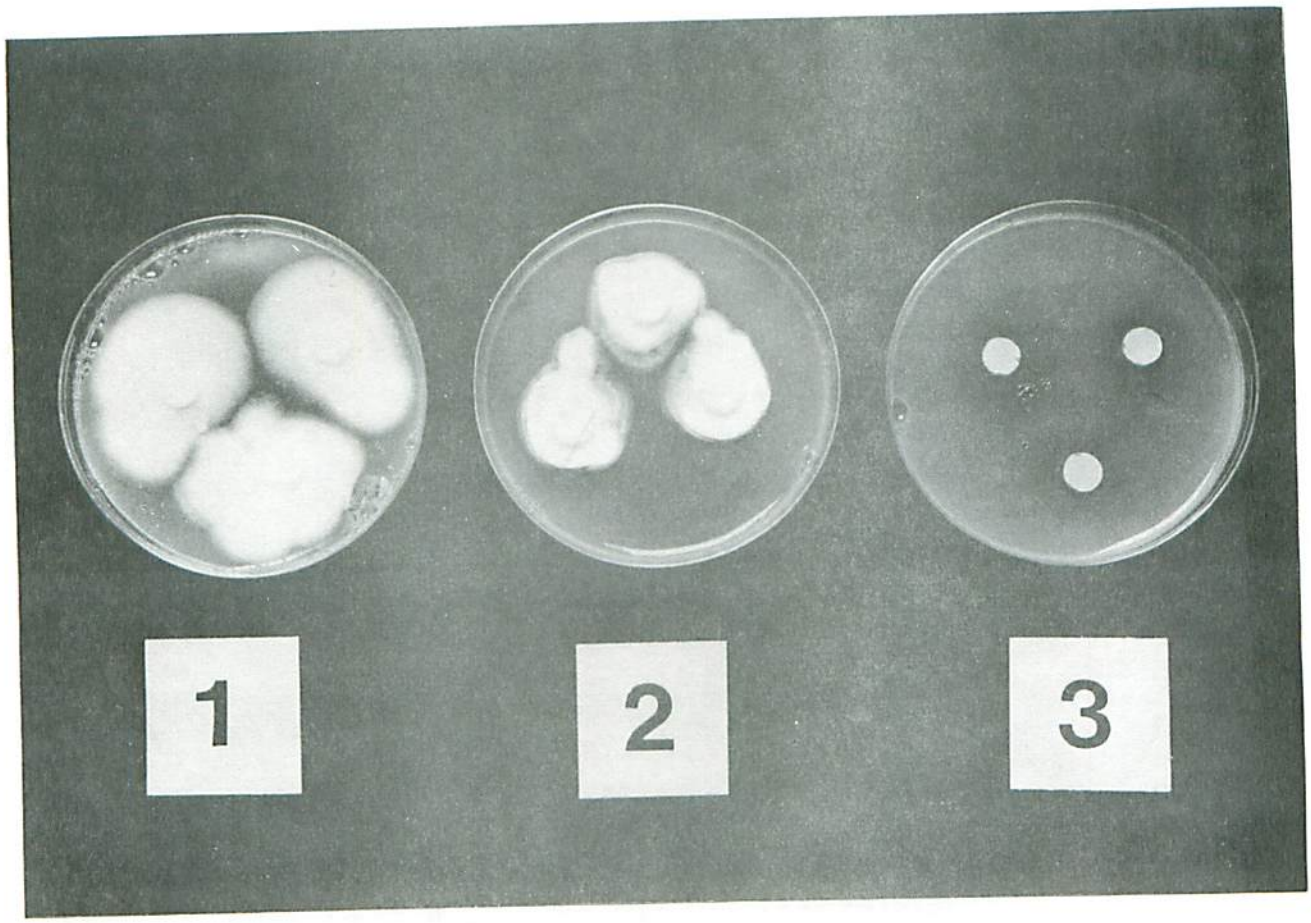

Fig. 6. Effect of a fungicide (Dowicide) on colony formation of DED. (1) control; (2) low concn.; (3) high concn. Note the total inhibition of growth at the high concentration.

SUMMARY AND CONCLUSIONS

Twenty chemical compounds of different chemical structure were screened for activity against the Dutch elm disease organism, Ceratocystis ulmi, (Buism) Moreau, under laboratory conditions using a modified agar technique. The rate of colony formation was inhibited by all materials at high concentrations, at 2, 4 and 6 days, but lower concentrations of some chemicals (Daconil and Cupramar) actually stimulated growth. Judging from the ED_{50}, the order of relative toxicity was as follows:- Lignasan > Acti-dione = Baydam 18654 = benomyl = MBC-Chloride > Dowicide-A > Du-ter > Calixin > Zyban > R-28921 = Validacin > PP3957 > Meltatox = Plondrel > Vitavax > Macuprax > Agro-Chem > Captan > Daconil > Cupramar. It is suggested that phytotoxicity and systemicity of some promising compounds should be tested in the greenhouse.

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APPENDIX

CHEMICAL NOMENCLATURE OF COMPOUNDS & THEIR SOURCES

Compound	Chemical Name	Source
1. Acti-Dione	(3-(2-(3,5-Dimethyl)-2-Hydroxyethyl)	Upjohn Chem. Co. (U.S.A.)
2. AG-GRO-CHEM	--	Kolmar of Canada Ltd.
3. Bay Dam 18654	Methyl (1-(5-Cyanopenty) (Amino Carbamoyl)(1-H-Benzimidazole) Carbamate	Chemagro Division (U.S.A.)
4. Benomyl	1-(Butylcarbamoyl)-2-Benzimidazole Carbamic acid, Methyl Ester	Dupont Canada Ltd.
5. Calixin	--	BASF
6. Captan Tech.	N-(Trichlormethyl)-4-Cyclohexane 1,2-Dicarboximide	Stauffer (U.S.A.)
7. Cupramar	Copper Oxychloride	Bharat Pulverizing Mills, India
8. Daconil	Chlorophalonic acid	Dept. of Agriculture (U.S.A.)
9. Dowicide-A	Sodium O-phenylphenoxide	Dow Chem. Co. (U.S.A.)
10. Du-Ter	--	Philips - Duphar (Holland)
11. Lignasan	MBC-HCl	DuPont Co. U.K.
12. Macuprax 2008	Basic Cupric Sulphate plus Cofraneb (An Ethylene Bis-Dithiocarbamate Complex of Zn, Mm, Fe, Cu)	McKechnie Chem. Ltd. U.K.
13. MBC-Chloride	1-(Butylcarbamoyl)-2-Benzimidazole Carbamic Acid, Methyl Esther	Laboratory Preparate
14. Meltatox	--	--
15. Plondrel	--	Dow Chem. Co. (U.S.A.)

16. PP 395	--	Chipman Chem. Co. (Canada)
17. R-28921	--	--
18. Validacin	Antibiotic Validamicyn A	Takeda Chem. Industries Ltd., Japan.
19. Vitavax Tech	--	Uniroyal Chem. Co. (Canada).
20. Zyban	50% Dimethyl 4, 4-O-Phenylenebis (3-Thioallophanate)	Mallincroft Chem. Co.