EVALUATION OF FUNGICIDES FOR CONTROL OF TREE DISEASES

III. Screening Against the Poplar Canker *Cytospora chrysosperma* (Pers.) Fr. Under Laboratory Conditions.

by

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RÉSUMÉ

On a éprouvé dix-neuf composés chimiques de structure et d'activité différentes pour leur effet contre le chancer cytosporéen, Cytospora chrysosperma (Pers.) Fr., en laboratoire, à l'aide d'une méthode modifiée de culture sur agar. Le taux de formation des colonies a été inhibé pour tous les traitements, à 2, 4 et 6 jours, et des concentrations plus faibles de certains composés ont au contraire activé la croissance (Plondrel et Ag-Gro-Chem). D'après la dose efficace des composés, réduisant de moitié le taux de formation des colonies (DE_{50}), leur toxicité relative s'échelonne comme suit: Acti-Dione = Bénomyl = Calixin = Dowicide A = Du-Ter = Lignasan = MBC-HCl = Meltatox = Zyban > Captan Tech = Daconil = Validacin > PP395 > R-28921 > Cupramar > Ag-Gro-Chem > Macuprax > Plondrel > Vitavax. Il est proposé d'etudier en serre la toxicité pour les plantes et la télétoxicité de certains composés prometteurs.

INTRODUCTION

Cankering of poplar by Cytospora chrysosperma has been recorded in North America and Europe since 1931 (Schreiner, 1931; Schmidle, 1953). Bier (1939) noted the presence of C. chrysosperma as a secondary pathogen on Russian poplars which had been attacked by Septoria musiva Peck. while Butin (1955) found that Populus deltoides Bartr. cuttings could be successfully inoculated with C. chrysosperma only if their moisture content was below a certain level. Thus, there is a danger of infection to poplar cuttings by this pathogen in the nurseries and therefore it is important to devise some control measures. Actually, according to Wright (1957) shelterbelts of P. deltoides are also susceptible to C. chrysosperma attack when growing in unfavourable moisture conditions. The purpose of the present investigation was therefore, to search for some effective chemicals that could inhibit the growth of this parasite under laboratory conditions and this report describes the action of 19 compounds of different chemical structure and activitiy on the rate of colony formation of C. chrysosperma.

MATERIALS AND METHODS

(i) Chemical Compounds

Samples of nineteen commercially available compounds, currently introduced into the market were used in these tests. The details of their chemical structures and description together with the name of chemcial 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 Cytospora chrysosperma fungi. 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 Cytospora chrysosperma (Pers.) Fr. were obtained from the Great Lakes Forest Research Center, Sault Ste. Marie, Ontario. The fungi 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 with the five concentrations: 0; ppm; 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^{o} 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. These plates were then temporarily stored in a refrigerator for future use. Subsequently the screening was done by placing three disks of pure Cytospora chrysosperma culture on each of two plates containing mixture of PDA and the candidate compound. In this way six replicates of each concentration were used to measure the growth response of the implanted fungus. Measurements were taken at 2,4, and 6 day intervals and the growth in each plate was compared to that of the controls. From a series of growth curves, the effective concentration halving the rate of colony formation (ED_{50}) at a time period was computed and this parameter was employed to compare relative potency of each candidate compound.

RESULTS AND DISCUSSION

The progressive growth of the pathogen in control and treated agar plates was measured at 2, 4 and 6 days. The dose-response curves for each compound are presented in Fig. 1, 2, 3, 4 and 5. As can be seen, some candidate chemicals are more potent than others. It seems 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 to note is the stimulatory effects of low concentration of some fungicides (Plondrel and Ag-gro-chem). 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 I. For better comparison, relative

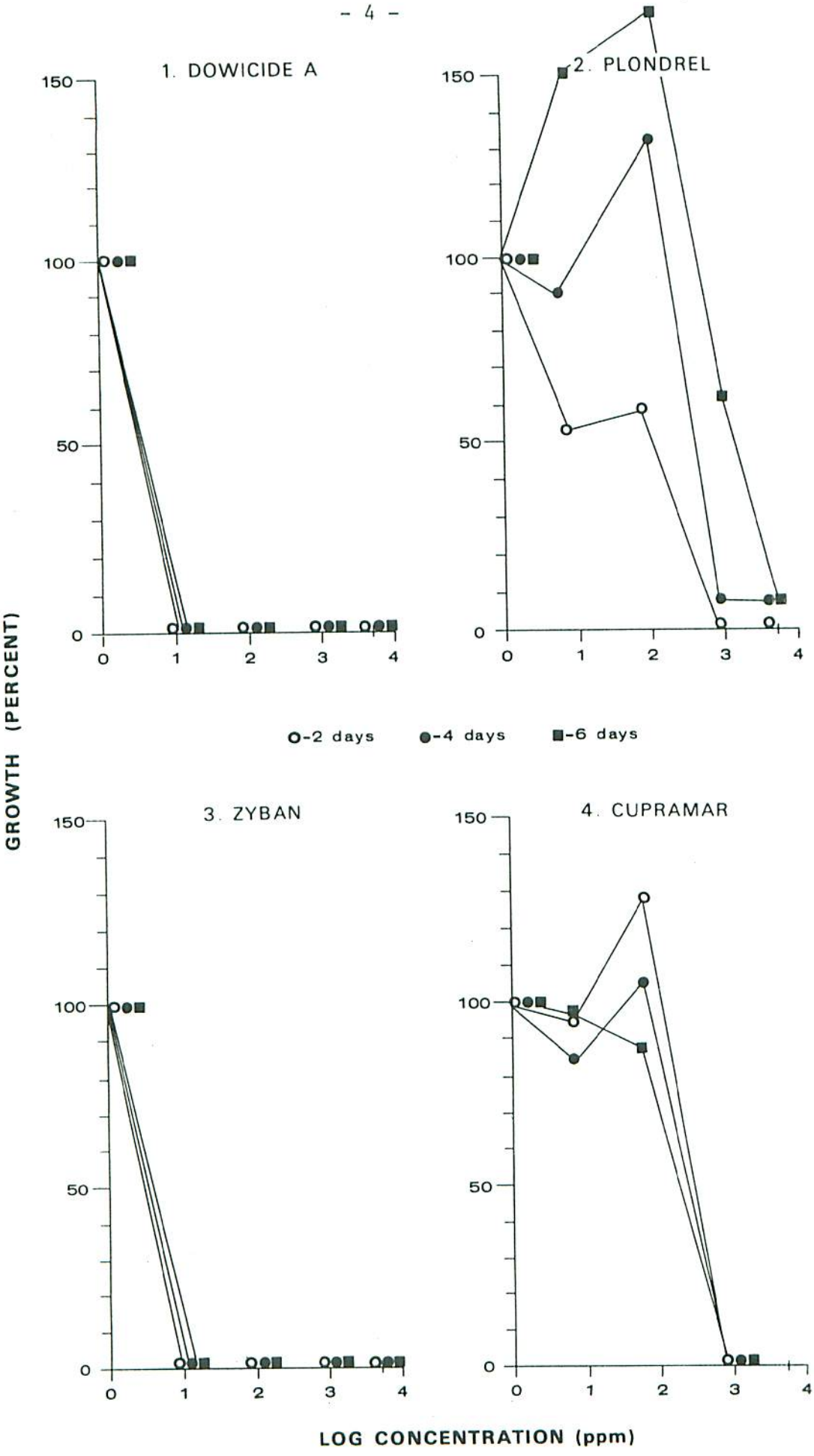

Fig. 1. Dose-response curves of Cytospora chrysosperma following treatment with Dowicide A, Plondrel, Zyban and Cupramar after 2, 4 and 6 days.

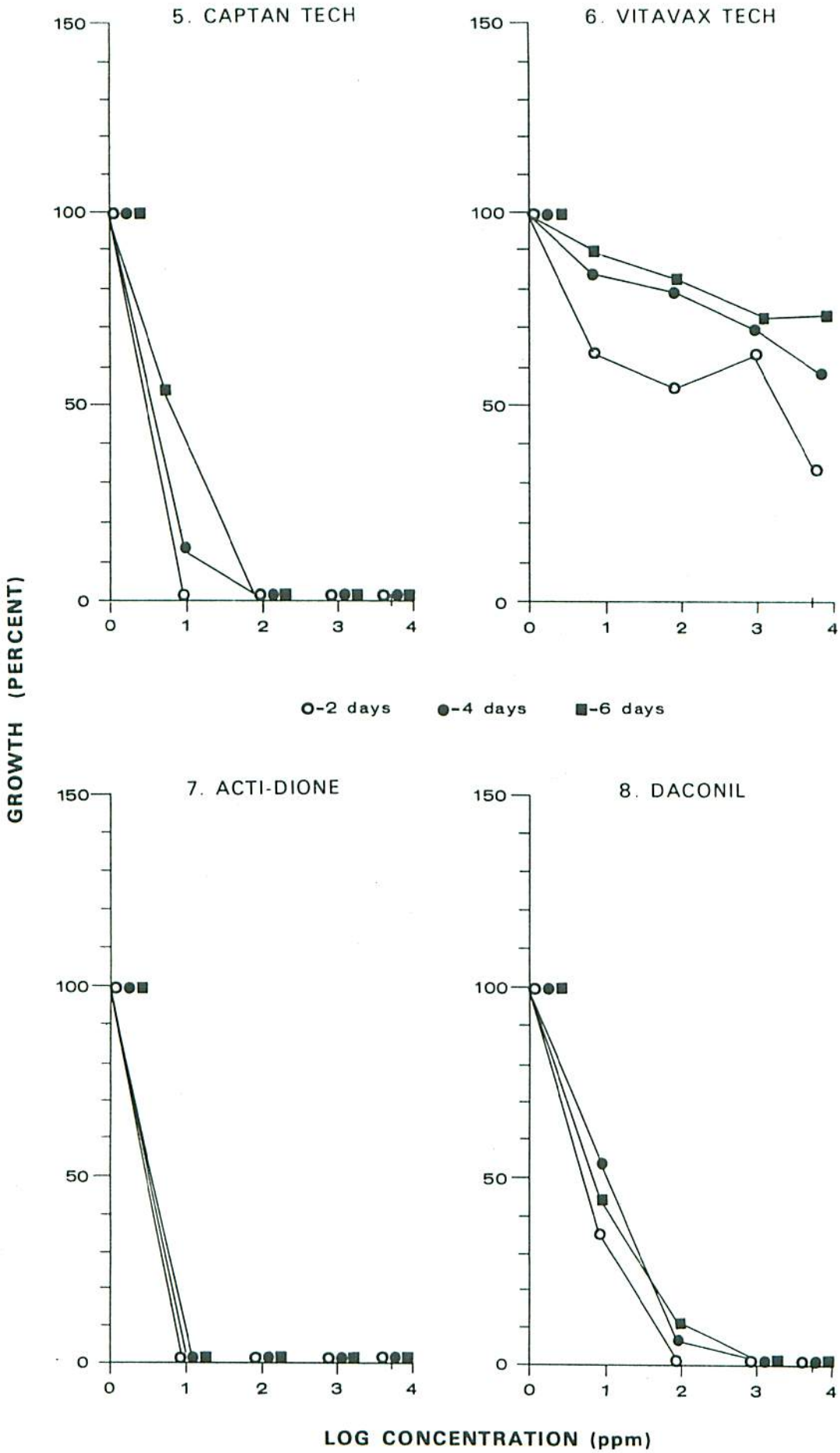

Fig. 2. Dose-response curves of Cytospora chrysosperma following treatment with Captan Tech, Vitavax Tech., Acti-Dione and Daconil after 2,4 and 6 days.

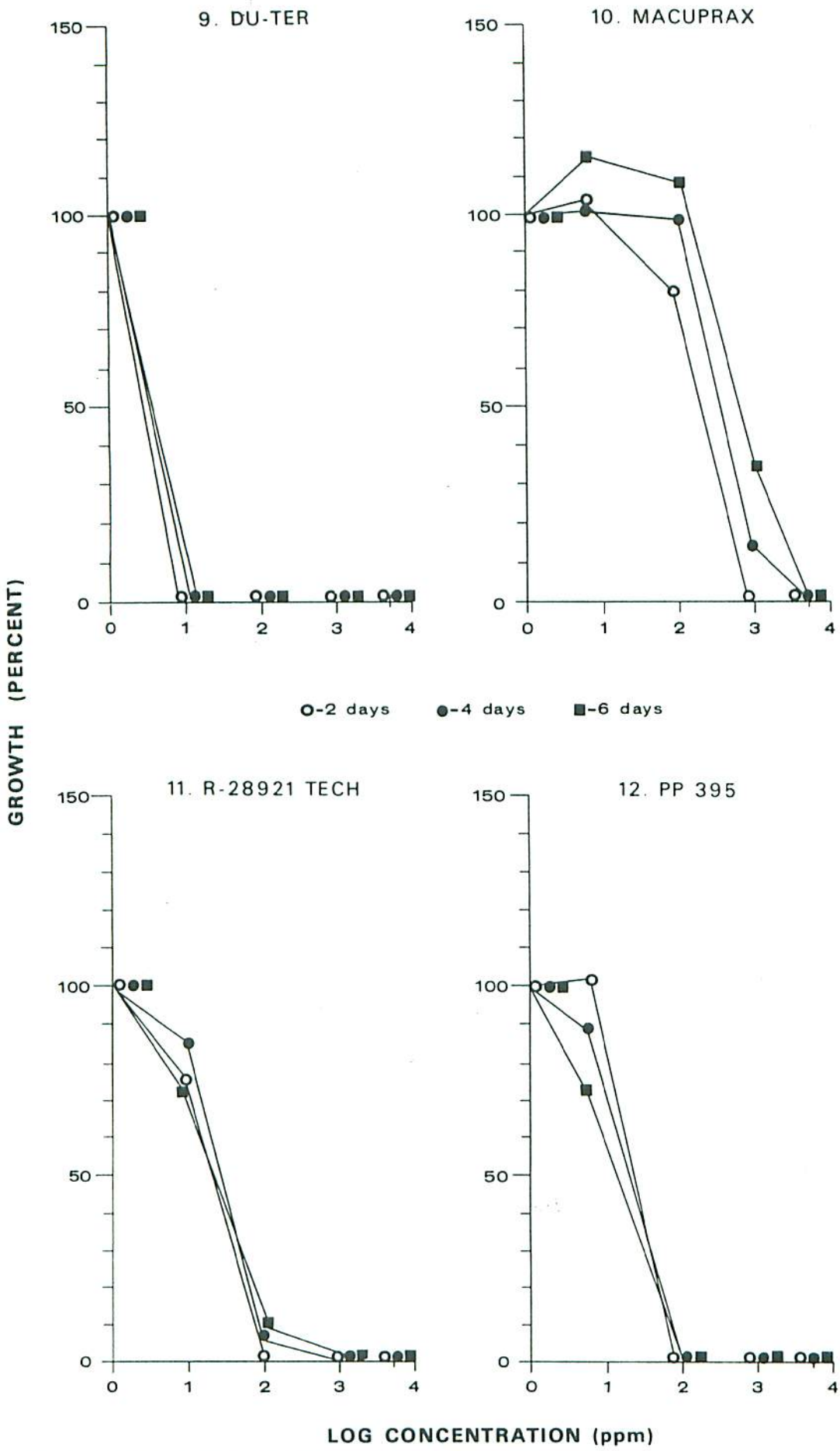

Fig. 3. Dose-response curves of Cytospora chrysosperma following treatment with Du-Ter, Macuprax, R-28921 Tech., and PP 395 after 2,4 and 6 days.

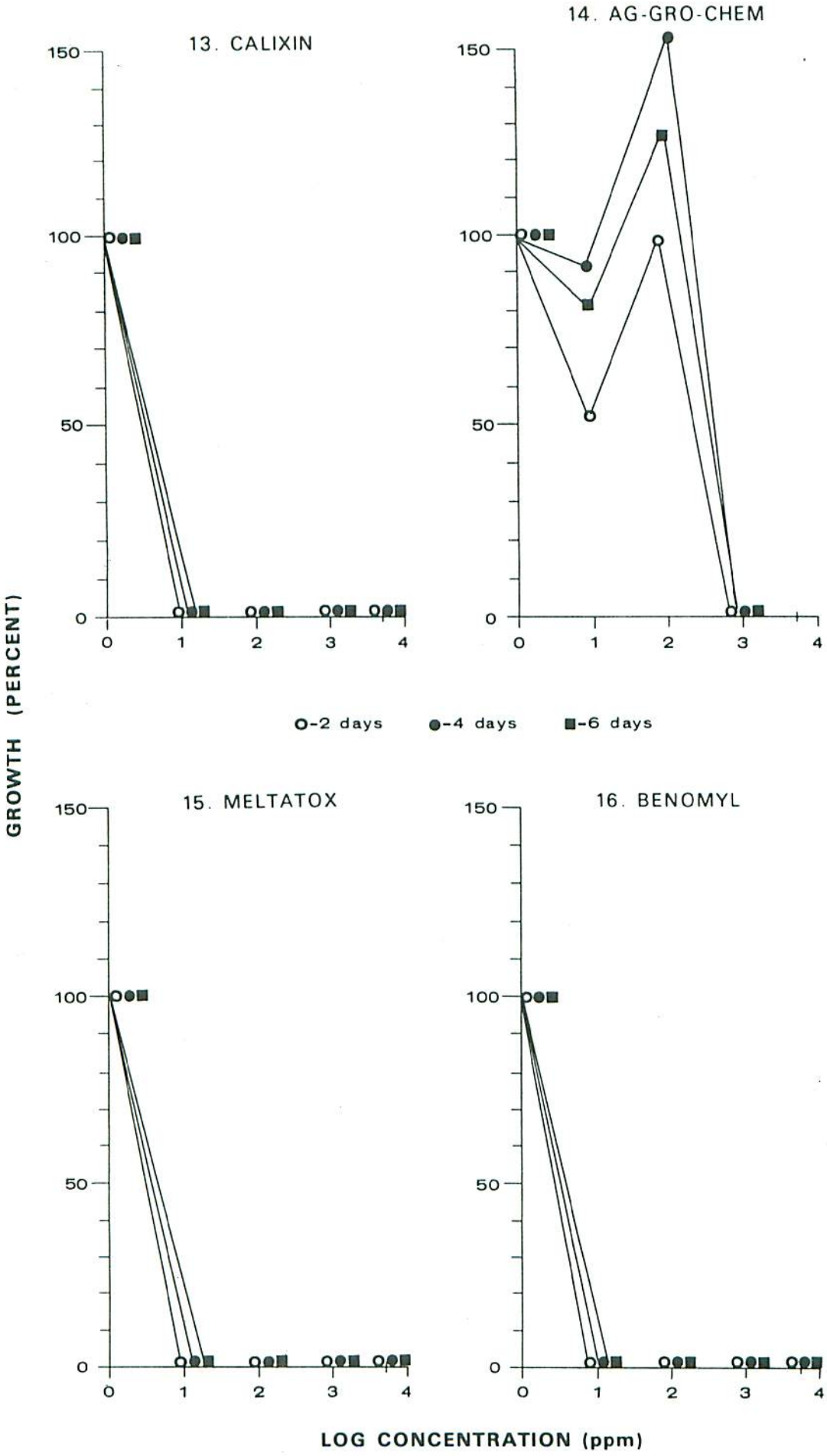

Fig. 4. Dose-response curves of Cytospora chrysosperma following treatment with Calixin, Ag-Gro-Chem, Meltatox and Benomyl after 2, 4 and 6 days.

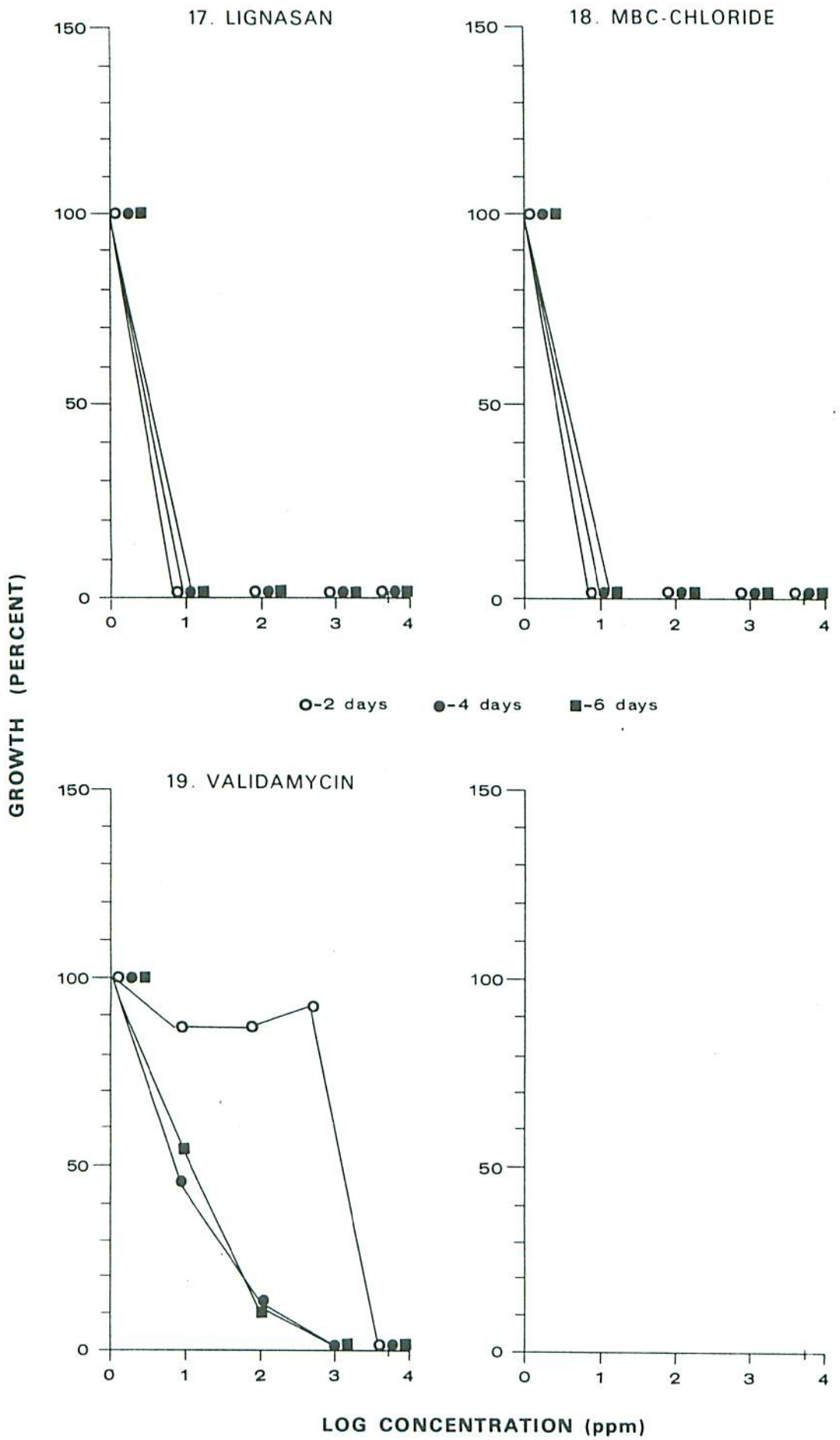

Fig. 5. Dose-response curves of Cytospora chrysosperma after treatment with Lignasan, MBC-Chloride and Validamycin after 2, 4 and 6 days.

inhibition and inhibition index were transferred from this data according to procedure outlined by Prasad and Travnick 1974. It seems the present method of mixing the fungicide directly with the agar and then implantation of Cytospora chrysosperma disks is superior to the previously used "well technique" (Prasad and Travnick 1972) because the fungicide had a change to uniformly distribute and diffuse throughout the agar medium. 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 ($100^{o}F$) 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 $100^{o}F$, 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 that the most fungitoxic compounds are: Acti-Dione, Benomyl, Calixin, Dowicide A, Du-Ter, Lignasan, MBC-HCl, Meltatox, Zyban, followed by Captan Tech., Daconil, Validacin, PP 395, R-28921 Tech., Cupramar, Ag-Gro-Chem and Macuprax. Similarly there were other compounds like Plondrel and Vitavax that were least effective and their use as fungitoxicants must be questioned. Acti-Dione, Benomyl, Calixin, Du-Ter, Dowicide A, Lignasan, MBC-HCl, Meltatox and Zyban can be regarded as potential fungicides for Poplar Canker 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 produce phytotoxic effects and as a consequence cannot be effectively employed as systemic material for the control of vascular diseases in forest trees.

TABLE I

Approximate ED_{50} - CYTOSPORA CHRYSOSPERMA (PERS.) Fr.

	Fungicide	ED_{50}	Relative Inhibition	Inhibition Index
1.	ACTI-DIONE	3 ppm	1.00	100
2.	BENOMYL	3	1.00	100
3.	CALIXIN	3	1.00	100
4.	DOWICIDE A	3	1.00	100
5.	DU-TER	3	1.00	100
6.	LIGNASAN	3	1.00	100
7.	MBC-HCl	3	1.00	100
8.	MELTATOX	3	1.00	100
9.	ZYBAN	3	1.00	100
10.	CAPTAN TECH.	10	0.30	30
11.	DACONIL	10	0.30	30
12.	VALIDAMYCIN	10	0.30	30
13.	PP 395	25	0.12	12
14.	R-28921 TECH.	30	0.10	10
15.	CUPRAMAR	300	0.01	1
16.	AG-GRO-CHEM	500	0.006	0.6
17.	MACUPRAX	800	0.004	0.4
18.	PLONDREL	2000	0.002	0.2
19.	VITAVAX	3000	0.001	0.1

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 nineteen compounds.

SUMMARY AND CONCLUSIONS

Nineteen chemical compounds of different chemical structure and activity were screened against the Poplar canker, Cytospora chrysosperma (Pers.) Fr. under the laboratory conditions using a modified agar technique. The rate of colony formation was inhibited by all treatments at 2, 4 and 6 days and lower concentrations of some chemicals (Plondrel and Ag-Gro-Chem) actually stimulated the growth. Judging from the ED_{50}, the order of relative toxicity was as follows: Acti-Dione = Benomyl = Calixin = Dowicide A = Du-Ter = Lignasan = MBC-HCl = Meltatox = Zyban > Captan Tech = Daconil = Validacin > PP395 > R-28921 > Cupramar > Ag-Gro-Chem > Macuprax > Plondrel > Vitavax. 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.	Benomyl	1-(Butylcarbamoyl)-2-Benzimidazole Carbamic acid, Methyl Ester	Dupont Canada Ltd.
4.	Calixin	--	BASF
5.	Captan Tech.	N-(Trichlormethyl)-4-Cyclohexane 1,2-Dicarboximide	Stauffer (U.S.A.)
6.	Cupramar	Copper Oxychloride	Bharat Pulverizing Mills, India
7.	Daconil	Chlorophalonic acid	Dept. of Agriculture (U.S.A.)
8.	Dowicide-A	Sodium O-phenylphenoxide	Dow Chem. Co. (U.S.A.)
9.	Du-Ter	--	Philips - Duphar (Holland)
10.	Lignasan	MBC-HCl	DuPont Co. U.K.
11.	Macuprax 2008	Basic Cupric Sulphate plus Cofraneb (An Ethylene Bis-Dithiocarbamate Complex of Zn, Mm, Fe, Cu)	McKechnie Chem. Ltd. U.K.
12.	MBC-Chloride	1-(Butylcarbamoyl)-2-Benzimidazole Carbamic Acid, Methyl Esther	Laboratory Preparate
13.	Meltatox	--	--

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