EVALUATION OF FUNGICIDES FOR CONTROL OF TREE DISEASES

V. - Screening Against the Dutch Elm Disease *Ceratocystis ulmi* (Buism) C. Moreau Under Laboratory and Field Conditions During 1976

by

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RÉSUMÉ

L'efficacité de vingt produits chimiques contre l'agent de la maladie hollandaise de l'orme, Ceratocystis ulmi (Buism) Moreau a été éprouvée en laboratoire à l'aide d'une technique à la gélose modifiée. La vitesse de formation des colonies a été mesurée après 2, 4 et 6 jours, et les doses (ED_{50}) de produits l'ayant réduite de moitié après une période de 6 jours ont été calculées. D'après ces résultats, les produits sont classés comme suit, par ordre décroissant de toxicité:

EL-222 E.C. > Hymexazol F-319 > Rovral LFA 2043
> Nystatin > Terraclor W.P. > P-588 > SN-43410
> Cela W-524 > Bay Meb-6447 > DPX-3217 > SN-43493
= F-7771 > Nabac 25 E.C. > Bay 406 1302 > SN-41703
> Terrazole > Terramycin > Polysul = SN-39744
= Chitinase.

Il est proposé de mesurer, en serre et sur le terrain, les propriétés phytotoxiques et endothérapiques des produits les plus prometteurs. Des études préliminaires de l'action endothérapique relative de 2 fongicides, dans les conditions réelles ont révélé une phytotoxicité nulle.

INTRODUCTION

The fungus *Ceratocystis ulmi* (Buism) C. Moreau, which causes the fatal Dutch elm disease in elm trees (*Ulmus americana* L.) across Canada and the United States could be suppressed by a fungicide, benomyl, and to a greater extent by derivatives of benomyl, Lignasan^{R}-phosphate or chloride (Prasad 1974, 1975). However one of the drawbacks of benomyl is its low solubility in water and the more soluble formulations such as Lignasan^{R}-P have acidic components which, at times, not only corrode the application equipment but precipitate out of solution.

In addition, it has been recorded by several investigators (Berger 1973; Litterel 1974; Clarke et al 1974) that repeated use of benomyl against pathogens induces resistance among them. Then again, benomyl has been found to be harmful to certain forest ecosystems (Prasad and Moody 1974). Therefore, there is a need to screen more and more newly developed fungicides against the Dutch elm disease pathogen.

With this objective in mind, during the summer of 1976, twenty compounds were evaluated in the laboratory and field and this report presents the relative effectiveness of these compounds in inhibiting the growth of Dutch elm disease pathogen under laboratory conditions, together with field evaluations of two previously laboratory screened pesticides on large elm trees for systemic and phytotoxic effects.

MATERIALS AND METHODS

(i) Chemical Compounds

Samples of twenty chemicals were selected, their solubility in water tested and the acidity (pH) of stock solutions measured. For better comparison, the strength of each compound was converted to a 100% active ingredient basis (Prasad and Travnick 1972; 1974; 1975). Stock solutions were then diluted to obtain the four concentrations used and a control (untreated) sample was run simultaneously for comparison.

(ii) Culture of Fungi

Vigorously growing, pure strains of *Ceratocystis ulmi* (Buism) C. Moreau were used, their pathogenicity first being tested on young elm trees. Strains were cultured in Petri dishes (100 x 15 mm) containing 15 ml of potato-dextrose-agar (PDA). From fully developed colonies, 10 mm diameter discs were cut out using a #7 cork borer, and transferred to a set of Petri dishes containing 15 ml of PDA treated without and with the tested fungicides at different concentrations. Strict aseptic conditions were maintained during the procedure.

(iii) Fungicide Treatment

The screening of each candidate fungicide was carried out at five concentrations: 0 ppm; 10 ppm; 100 ppm; 1000 ppm and 500 ppm. A sterile PDA medium was prepared in an autoclave and when the agar had cooled down to about 38^{o}C, a required amount of the fungicide was added to 30 ml of agar in a beaker and divided equally between two Petri dishes, thus each containing 15 ml of medium 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 discs 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 inhibition were taken at 2, 4 and 6 day intervals and the growth in each plate was compared to that of the controls (Prasad and Travnick 1974; 1975). 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 used to compare relative potency of each screened compound.

(iv) Field Testing

In late summer of 1976 two promising fungicides, DOVICIDE A and DEMOSAN, which were previously screened in the laboratory, were tested at Shirley's Bay, Ottawa, on mature elm trees, using the portable trunk-injection apparatus, developed by the Chemical Control Research Institute (Prasad 1975). Both fungicides were tested at 500 ppm concentration for distribution throughout the trees and for phytotoxicity. Sampling was done 15 and 30 days after the treatment. Because of dry weather the uptake of solution slowed considerably.

RESULTS AND DISCUSSION

The dose-response curves for each compound are presented in Figs. 1, 2, 3, 4 and 5 and from inspection it is evident that some fungicides 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 response. Of interest is the stimulating effect of some compounds (Chitinase, Polysul, SN-39744). The most

effective fungicides were those of stock solution range of 5 to 6 pH. 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 inhibition and inhibition index were derived from these data according to the procedure outlined by Prasad and Travnick (1972). The comparative toxicity chart (Table I) shows that EL-222 E.C. was the most effective, followed closely by Hymexazol F-319, Rovral LFA 2043, Nystatin and Terraclor W.P. The promising compounds should be tested further for systemicity and phytotoxicity in the greenhouse and field, using trunk injection method.

In a preliminary testing of 2 compounds, Dovicide A and Demosan, that were screened last year and were found to be extremely potent against the DED under laboratory conditions, were injected into large elm trees under field conditions at Shirley's Bay. The translocationability of these compounds was then monitored two weeks and one month after the injection by the standard bioassay technique (Table II); at the same time trees were also observed for phytotoxicity symptoms (Table III). Complete lack of translocation might have resulted from inappropriate time of injection or from insensitivity of the bioassay technique to these two fungicides. More research is needed to clarify these aspects.

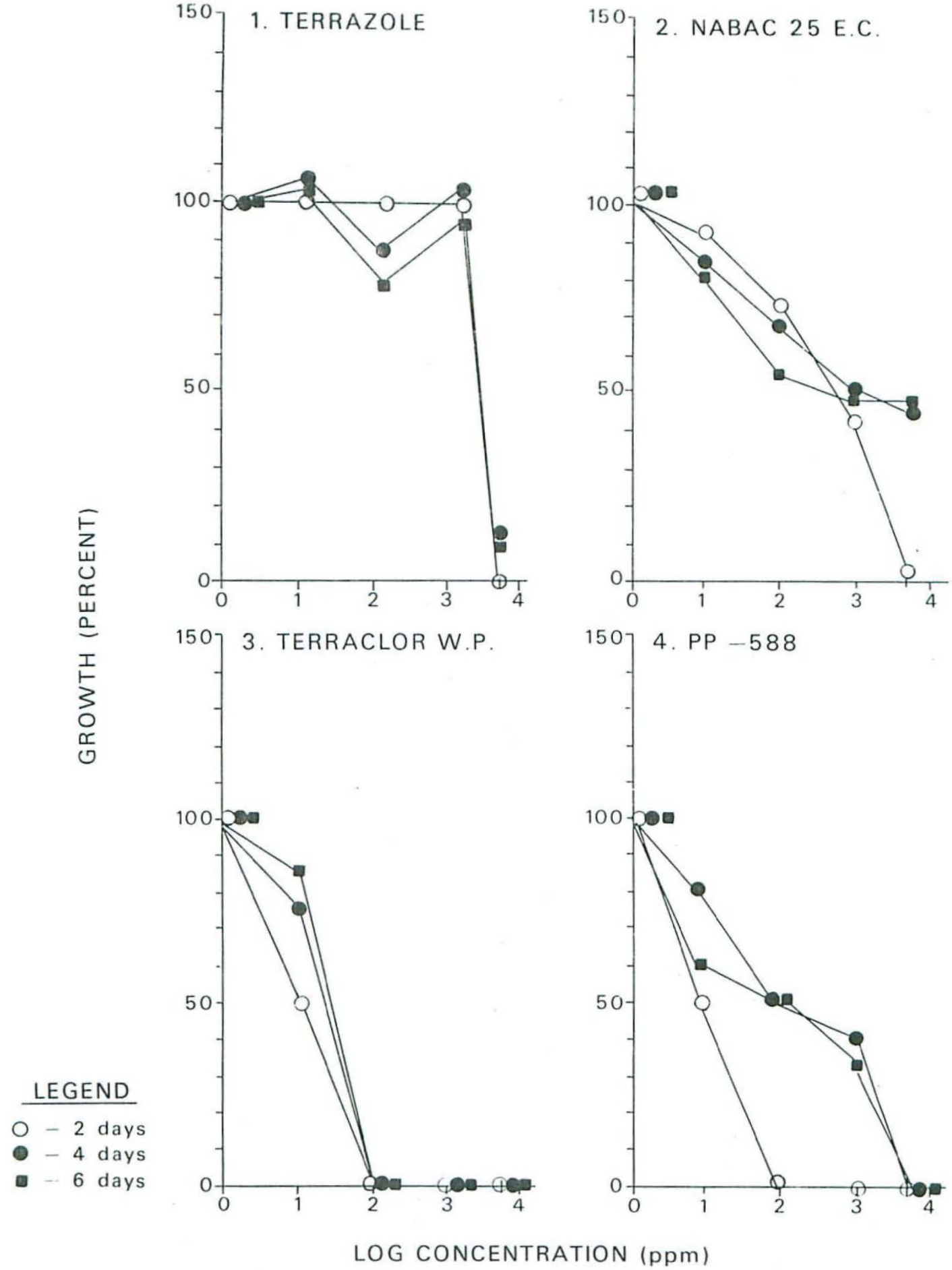

Fig. 1. Dose-response curves of *Ceratocystis ulmi* to treatment with Terrazole, Nabac 25 E.C., Terraclor W.P. and PP-588 for 2. 4 and 6 days.

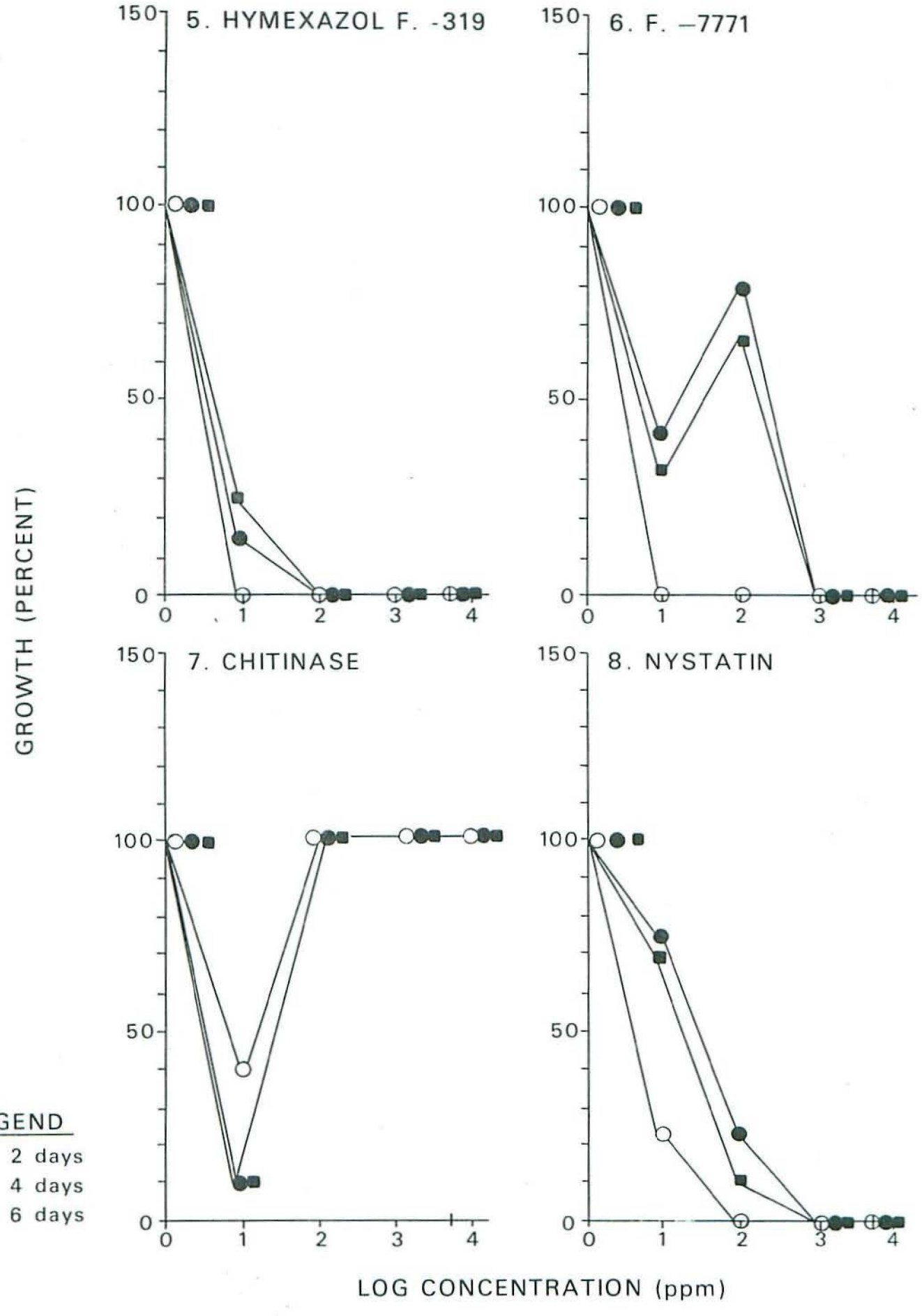

Fig. 2. Dose-response curves of *Ceratocystis* sp. to treatment with Hymexazol F-319, F-7771, Chitinase and Nystatin for 2, 4, and 6 days.

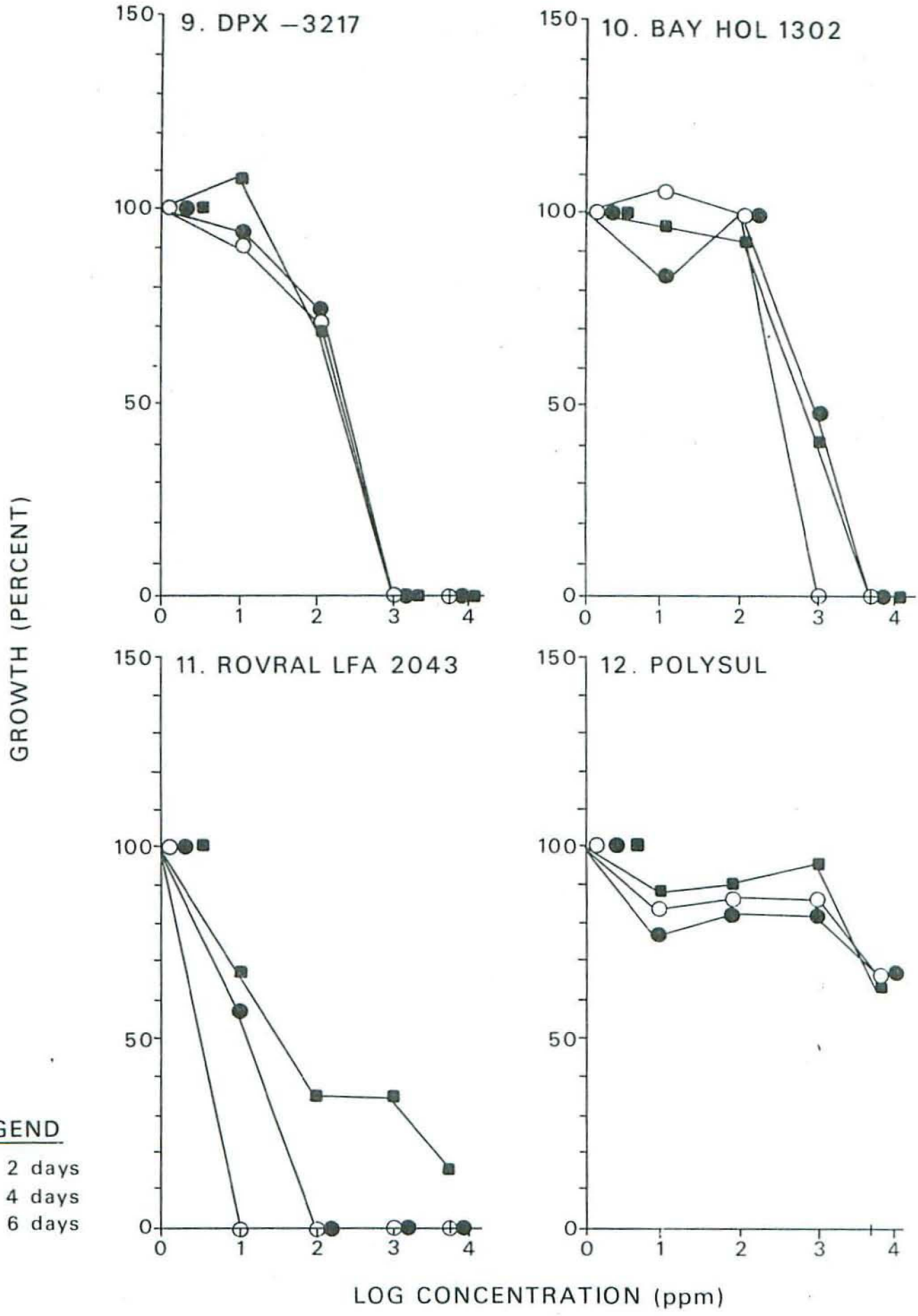

Fig. 3. Dose-response of *Ceratocystis ulmi* to treatment with DPX-3217, Bay Hol 1302, Rovral LFA 2043 and Polysul for 2, 4 and 6 days.

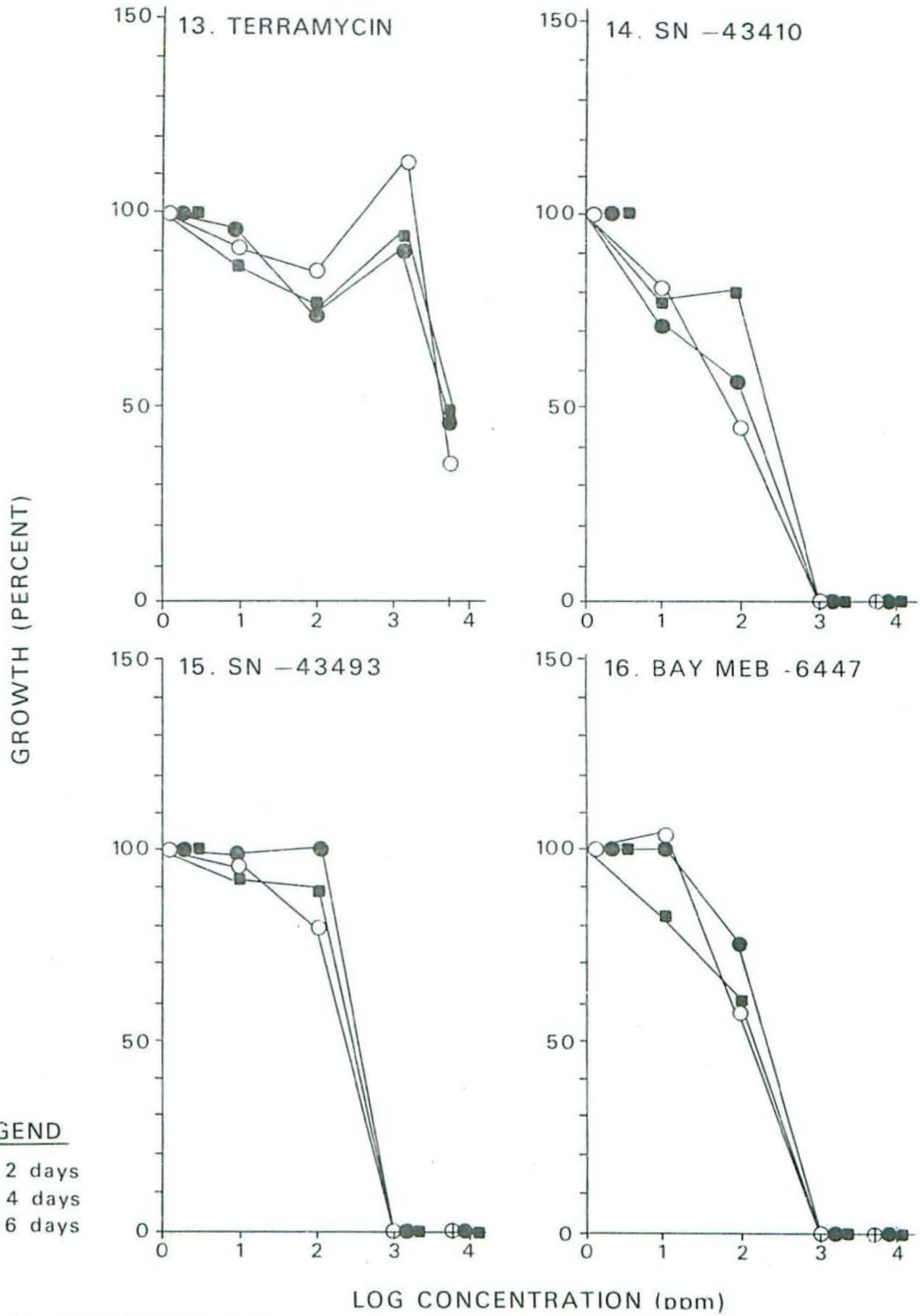

Fig. 4. Dose-response of *Ceratocystis ulmi* to treatment with Terramycin, SN-43410, SN-43493 and Bay Meb-6447 for 2, 4 and 6 days.

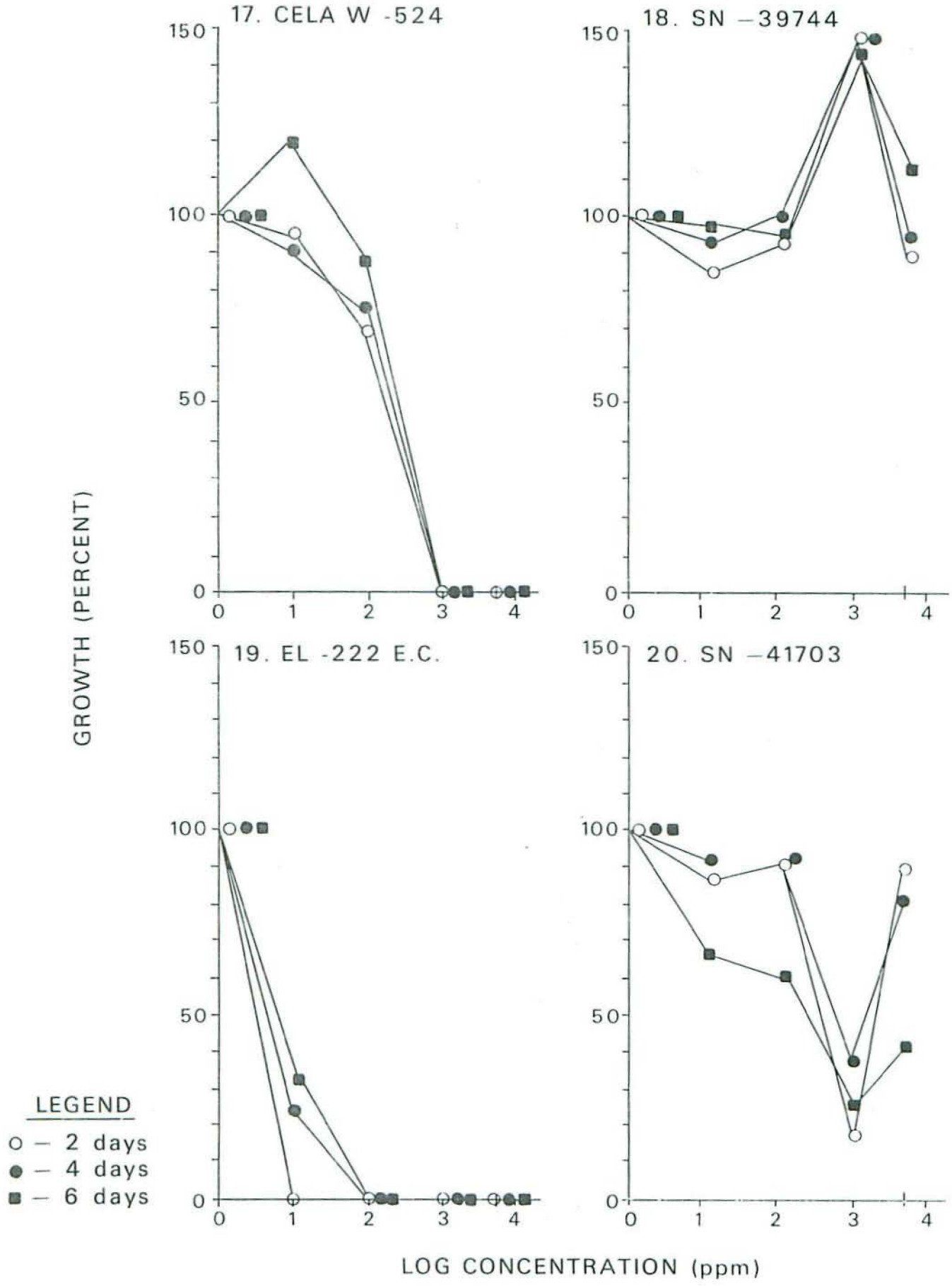

Fig. 5. Dose-response of *Ceratocystis ulmi* to treatment with Cela W-524, SN-39744, EL-222 E.C. and SN-41703 for 2, 4 and 6 days.

Table I

Comparative Toxicity of Compounds Tested Against *Ceratocystis ulmi* After Six Days

	Fungicide	ED_{50} (ppm)	Relative Inhibition	Inhibition Index
1.	EL-222 E.C.	6	1.00	100
2.	HYMEXAZOL F-319	7	0.86	86
3.	ROVRAL LFA 2043	10	0.60	60
4.	NYSTATIN	15	0.40	40
5.	TERRACLOR W.P.	25	0.24	24
6.	PP - 588	100	0.06	6
7.	SN - 43410	150	0.04	4
8.	CELA W - 524	170	0.03	3
9.	BAY MEB - 6447	180	0.03	3
10.	DPX - 3217	250	0.02	2
11.	SN - 43493	300	0.02	2
12.	F -7771	300	0.02	2
13.	NABAC 25 E.C.	700	0.01	1
14.	BAY HOL 1302	900	0.007	0.7
15.	SN - 41703	1000	0.006	0.6
16.	TERRAZOLE	3500	0.002	0.2
17.	TERRAMYCIN	5000	0.001	0.1
18.	POLYSUL	--	--	--
19.	SN - 39744	--	--	--
20.	CHITINASE	--	--	--

Table II

Distribution of fungicides in mature elm trees following trunk-injection after two and four weeks*

Fungicide	Concentration (ppm)	Leaves		Twigs	
		2 weeks	4 weeks	2 weeks	4 weeks
DOVICIDE A	500	0	0	0	0
DEMOSAN	500	0	0	0	0

* mean of 3 trees.

Table III

Effects of trunk-injection of fungicides on foliar symptoms in mature elm trees after two and four weeks *

Fungicide	Concentration (ppm)	Symptoms after 2 weeks	4 weeks
DOVICIDE A	500	NO EFFECT	NO EFFECT
DEMOSAN	500	NO EFFECT	NO EFFECT

* Mean of 3 trees.

SUMMARY

Twenty chemical compounds of different chemical structures were screened for activity against the Dutch elm disease pathogen, *Ceratocystis ulmi* (Buism) Moreau, under laboratory conditions, using a modified agar technique. The rate of colony formation was measured at 2, 4 and 6 days and the concentrations halving the growth rate (ED_{50}) at 6 day-period are computed. Judging from the ED_{50}, the order of relative toxicity was as follows:- EL-222 EC. > Hymexazol F-319 > Rovral LFA 2043 > Nystatin > Terraclor W.P. > P-588 > SN-43410 > Cela W-524 > Bay Meb-6447 > DPX-3217 > SN-43493 = F-7771 > Nabac 25 E.C. > Bay 406 1302 > SN-41703 > Terrazole > Terramycin > Polysul = SN-39744 = Chitinase.

It is suggested that the phytotoxicity and systemicity of the more promising compounds should be tested in the greenhouse and field. Preliminary studies on testing of relative systemic action of 2 screened fungicides under field conditions showed that there were no phytotoxic effects.

ACKNOWLEDGEMENTS

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APPENDIX A

Hydrogen Ion Concentration (pH) of Stock Solutions of Experimental Compounds

	Name	Phase	pH
1.	BAY HOL 1302	Liquid	2.2
2.	BAY MEB 6447	Powder	5.0
3.	CELA W 524	Liquid	3.5
4.	CHITINASE	Powder	6.0
5.	DPX 3217	- " -	6.8
6.	EL-222 E.C.	Liquid	5.0
7.	F - 7771	- " -	9.8
8.	HYMEXAZOL F 319	- " -	5.9
9.	NABAC 25 E.C.	- " -	3.8
10.	NYSTATIN	Powder	5.5
11.	POLYSUL	Liquid	9.5
12.	PP 588	- " -	6.4
13.	ROVRAL LFA 2043	Powder	6.1
14.	SN 3974	Liquid	10.1
15.	SN 41703	- " -	4.2
16.	SN 43410	- " -	3.4
17.	SN 43493	- " -	2.3
18.	TERRACLOR W.P.	Powder	8.2
19.	TERRAMYCIN	- " -	2.7
20.	TERRAZOLE	Liquid	4.5

APPENDIX B

Chemical Nomenclature of Compounds and Their Source

	COMPOUND	CHEMICAL NAME	SOURCE
1.	Bay Hol 1302	2-Chlorothanesulfinic acid	Chemagro, U.S.A.
2.	Bay Meb 6447	1-(4-Chlorophenoxy)-3,3-dimethyl-1--(1H-1,2,4-triazol-1-yl/-2-butanone	" "
3.	Cela W 524	---	Niagara Chemicals Ltd., Canada
4.	Chitinase	Enzyme	Canada Packers Ltd., Canada
5.	DPX 3217	2-Cyano-N-(ethylaminocarbomyl)-2-(methoxyimino) acetamide	E.I. Dupont Co., U.S.A.
6.	El-222 E.C.	α-(2-chlorophenyl)-α-(4-chlorophenyl)--5-pyrimidinemethanol	Elanco Products Co., U.S.A.
7.	F-7771	S, triazine, 1, 3, 5-trimethyl-hexahydro	R.T. Vanderbilt Co., U.S.A.
8.	Hymexazol F319	3-hydroxy-5-methylisoxazole	Sankyo Co. Ltd., Japan.
9.	Nabac 25 E.C.	2,2'-methylene bis (3,4,6-trichlorophenol)	Kalo Laboratories Inc., U.S.A.
10.	Nystatin	anti-biotic	Cyanamid of Canada Ltd., Canada

APPENDIX B (CONT'D.)

11.	Polysul	Calcium polysulphide and thiosulphate	Chas. H. Lilly Co., U.S.A.
12.	PP 588	5-butyl-2-ethylamino-6-methylpyrimidin -4 yl dimethylsulphamate	Chipman Chem. Ltd., Canada
13.	Rovral LFA 2043	Glycophene	May & Baker (Canada) Ltd., Canada
14.	SN 39744	Propyl-N-γ-dimethylaminopropyl) carbamate	Nor-Am Inc., U.S.A.
15.	SN 41703	N-(3-dimethylaminopropyl)-thiocarbamic acid S-ethylester hydrochloride	" " "
16.	SN 43410	---	" " "
17.	SN 43493	---	" " "
18.	Terraclor W.P.	---	Olin Corp., U.S.A.
19.	Terramycin	oxytetracycline HCl	Pfizer Chemical Div., Canada
20.	Terrazole	---	Olin Corp., U.S.A.