EVALUATION OF FUNGICIDES FOR CONTROL OF TREE DISEASES

IV. - Screening Against the Dutch Elm Disease *Ceratocystis ulmi* (Buism) C. Moreau Under Laboratory and Field Conditions During 1975

by

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RESUMÈ

Vingt produits chimiques ont été évalués en laboratoire et à l'aide d'une technique modifiée employant la gélose comme milieu de culture, pour leur activité contre le *Ceratocystis ulmi* (Buism) Moreau, l'agent de la maladie hollandaise de l'orme. On a mesuré les vitesses de formation des colonies aprés 2, 4 et 6 jours, et calculé les concentrations capables de réduire de moitié le taux de croissance aprés 6 jours. Prenant pour point de départ le DE_{50} (dose efficace réduisant le taux de croissance de moitié), l'ordre de toxicité relative a été établi comme suit:

Lignasan-HCl = Busan 70 > Busan 30 - 1> BAS 3460F> Demosan > Orthocide > Dowco 263 > Phaltan > Isobac 20 > Dyréne > Terraclor Super X > Dithane M-45 > Dodemorph > Plantvax > SN 513 > Nurelle = Delan > Dowco 269 = Agrimycin 17 = Ferbam .

Il est proposé d'éprouver, dans des serres et sur le terrain, la toxicité à l'égard des plantes et télétoxicité des composés les plus prometteurs. Des études préliminaires sur le terrain portant sur la télétoxicité relative de six fongicides éprouvés ont montré qu'un seul s'est distribué dans les tissus végétaux alors que les autres étaient inefficaces ou toxiques à l'égard des feuilles.

INTRODUCTION

The beautiful elm trees (*Ulmus americana* L.) on the North American continent are being gradually decimated by the Dutch elm disease (DED), caused by the fungus *Ceratocystis ulmi* (Buism) Moreau. A few years ago, a new fungicide, benomyl, was developed by DuPont Chemical Co., U.S.A. (Delp and Klopping 1968), which proved effective against this pathogen. However, a serious drawback of this fungicide is its low solubility in aqueous formulations. The solubility increases in acid solvents, but too much acidity (below pH 2) is detrimental to the host tissue. Also, like other fungi, DED may eventually become resistant to benomyl and its derivatives (Berger 1973; Litterel 1974; Clarke et al 1974). There is yet another disadvantage of benomyl fungicides in the sense that they are hazardous to certain forest ecosystems (Prasad and Moody 1974). Thus there is a need to screen for other fungicides developed more recently.

Therefore, during 1975, a group of twenty compounds was screened against the fungus *Ceratocystis ulmi*; the objective being to discover a fungi-toxic compound of greater solubility in water and least hazardous to the environment. The present report describes the relative toxicity of twenty materials on the rate of colony formation of DED under laboratory conditions together with evaluation of six screened compounds on large and mature elm trees for systemic and phytotoxic effects.

MATERIALS AND METHODS

(i) Chemical Compounds

Samples of twenty fungicides were selected for these tests, with regard to their solubility. The chemical names of these compounds and the names of chemical companies supplying them are included in the appendix. Each fungicide was tested on the basis of a 100% active ingredient (Prasad and Travnick 1972 and 1974). The pH of the stock solutions was measured and appropriate dilutions were then made to obtain the range of the four concentrations used. A control (untreated) sample was also simultaneously run for comparison.

(ii) Culture of Fungi

Only pure and vigorously growing strains of *Ceratocystis ulmi* (Buism) Moreau were used; their pathogenecity were first tested on young elm trees. Re-isolates were then cultured in standard Petri dishes (100 x 15 mm) containing 15 ml of potato-dextrose-agar (PDA). When colonies in these plates were fully developed, discs of a 10 mm diameter were cut out with a # 7 cork borer and transferred, under aseptic conditions, to a set of Petri dishes containing 15 ml of PDA treated without and with fungicides at different concentrations.

(iii) Fungicide Treatment

The screening of each candidate fungicide was carried out at 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 the fungicide was added to a 30 ml of agar in a beaker and divided equally between two 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 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). 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

During the summer of 1975,a group of six fungicides, which were previously screened in laboratory against *Ceratocystis ulmi* with promising results, were trunk-injected into large elm trees under field conditions in Dechenes, using a portable hand-pump injection apparatus, developed by the Chemical Control Research Institute (Prasad, Travnick and Moody 1975).

Each fungicide was tested at two concentrations (1,000 ppm and 5,000 ppm) and sampled after two days and two months for systemic property.

Unfortunately there was a very dry spell at the time of injection and thus the uptake of fungicides by the trees was slowed down, and in some instances ceased altogether. As a consequence, some bioassays of sampled leaves and twigs didn't show much translocation of the fungicides.

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 fungicides (Dowco 269, Ferbam, Agrimycin) and increased potency with time of others (BAS 3460F, Orthocide, Phaltan). 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 Lignasan-HCl and Busan 70 were equally effective, followed closely by Busan 30-I, BAS 3460F, Demosan and Orthocide. 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 6 compounds, (Vancide TH, Vancide 40, SN 513, Validamycin, Dowicide-A, Terraclor-Super X) that were screened last two years and were found to be extremely potent against the DED under laboratory conditions, two concentrations (1000, 5000 ppm) of each were injected into large elm trees under field conditions at Deschenes. The translocability of these compounds was then monitored one week after the injection by the standard bioassay technique. Even though most of the samples did not show any positive bioassay response, many injected trees showed varying degrees of phytotoxicity (see Table II). Thus it appeared that Vancide-40, Dowicide, Terraclor-Super X and SN 513 would not be suitable as systemic fungicides at these concentrations.

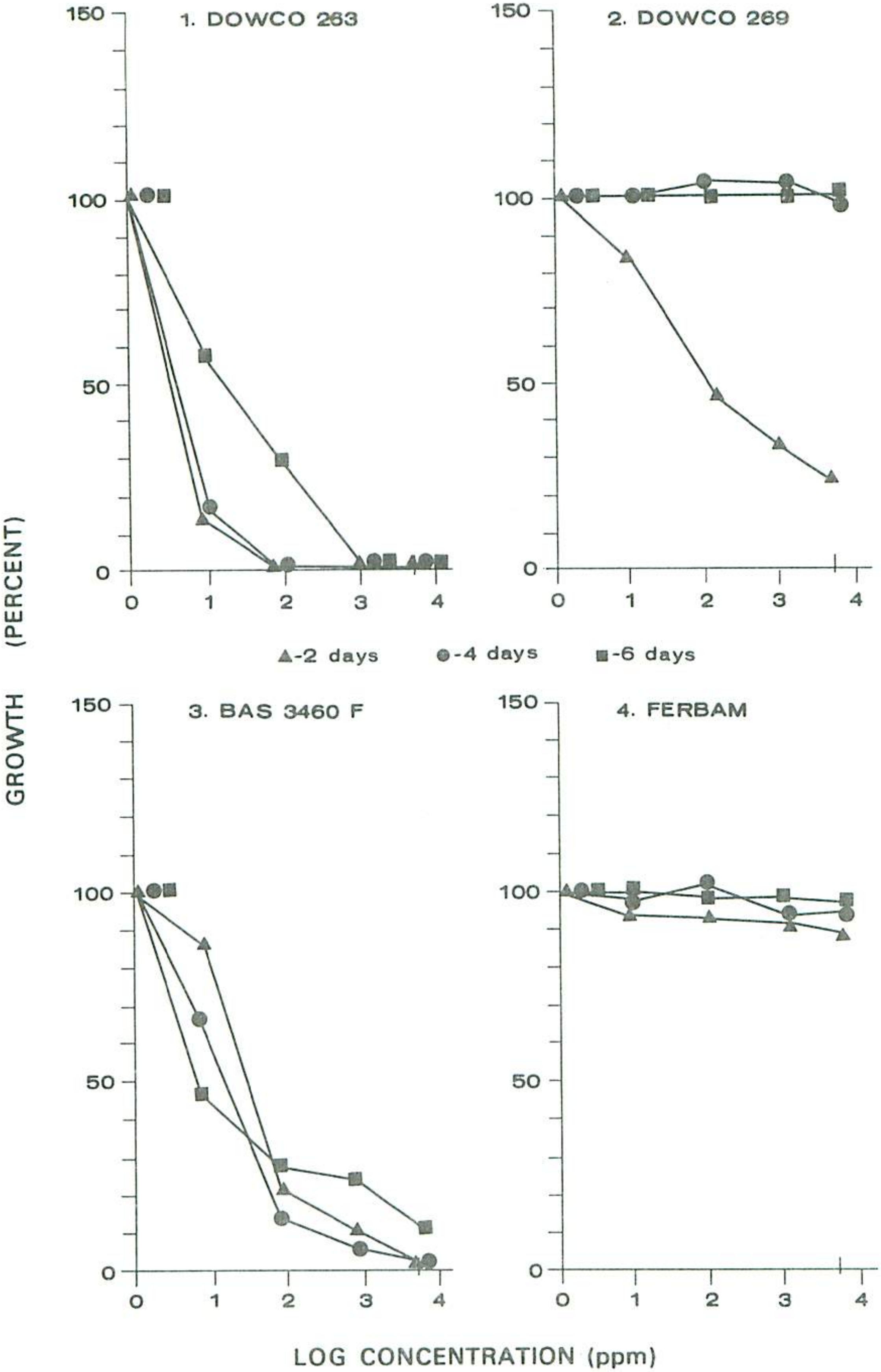

Fig. 1. Dose-response curves of _Ceratocystis ulmi_ to treatment with Dowco 263, Dowco 269, Bas 3460 F and Ferbam, for 2, 4 and 6 days.

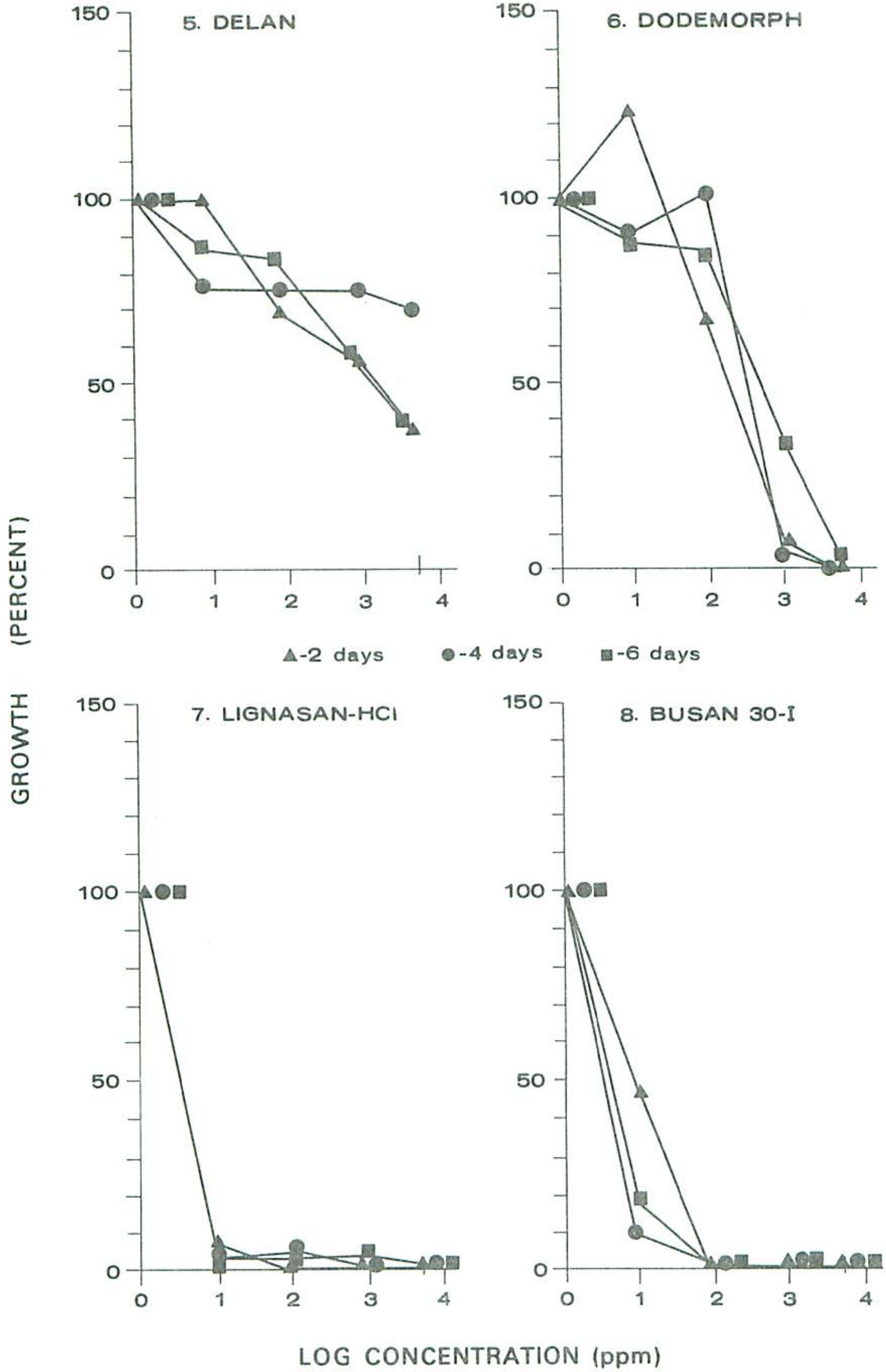

Fig. 2. Dose-response curves of Ceratocystis ulmi to treatment with Delan, Dodemorph, Lignasan-HCl and Busan 30-I, for 2, 4 and 6 days.

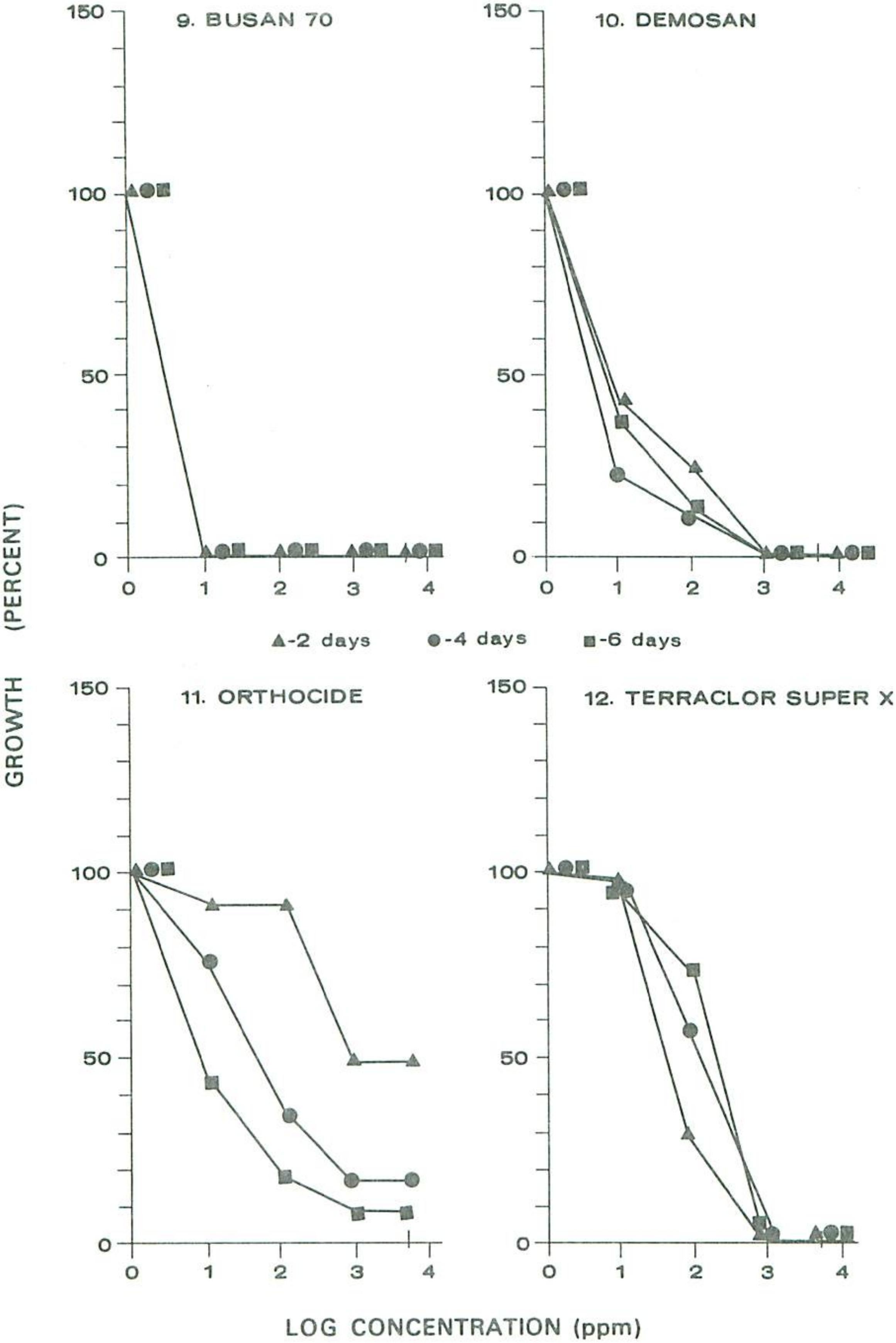

Fig. 3. Dose-response of Ceratocystis ulmi to treatment with Busan 70, Demosan, Orthocide and Terraclor Super X, for 2, 4 and 6 days.

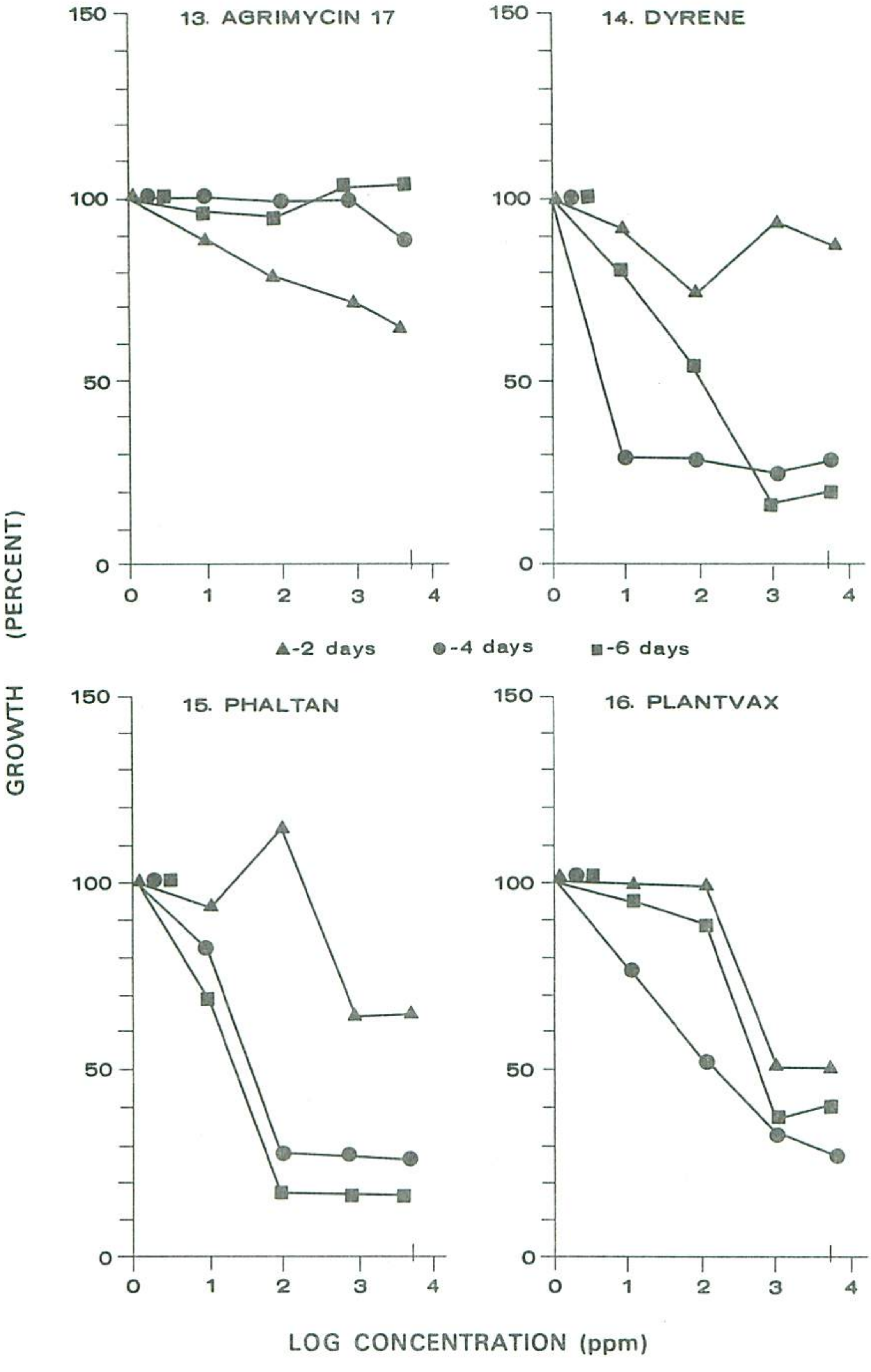

Fig. 4. Dose-response of Ceratocystis ulmi to treatment with Agrimycin 17, Dyrene, Phaltan and Plantvax, for 2, 4 and 6 days.

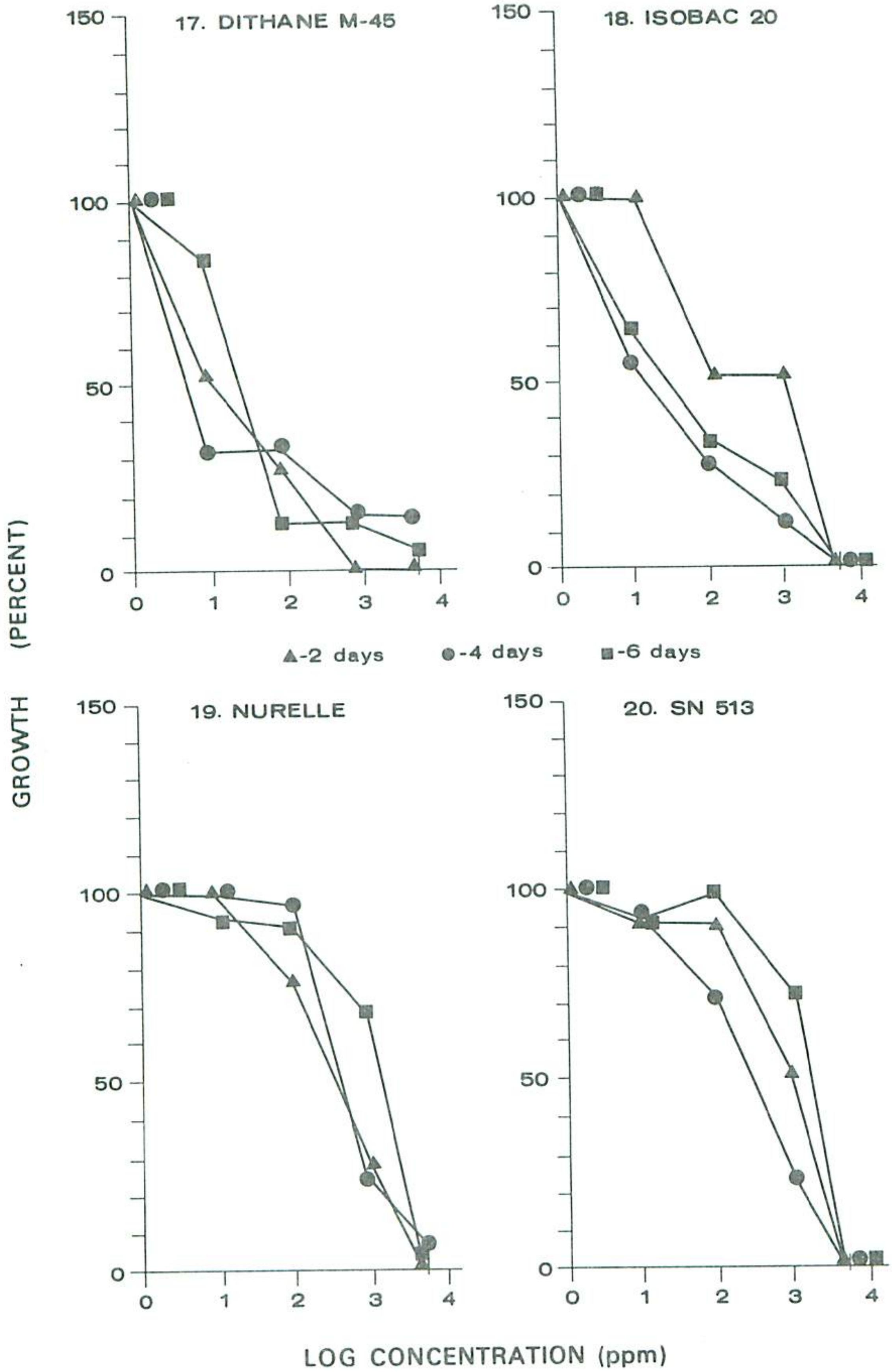

Fig. 5. Dose-response of <u>Ceratocystis ulmi</u> to treatment with Dithane M-45, Isobac 20, Nurelle and SN 513, for 2, 4 and 6 days.

TABLE I

Comparative Toxicity of Compounds Tested against
Ceratocystis ulmi After Six Days

Fungicide		ED_{50} (ppm)	Relative Inhibition	Inhibition Index
1.	Lignasan-HCl	3	1.00	100
2.	Busan 70	3	1.00	100
3.	Busan 30-I	4	0.75	75
4.	BAS 3460 F	6	0.50	50
5.	Demosan	8	0.38	38
6.	Orthocide	9	0.33	33
7.	Dowco 263	20	0.15	15
8.	Phaltan	25	0.12	12
9.	Isobac 20	30	0.10	10
10.	Dyrene	100	0.03	3
11.	Terraclor Super X	200	0.02	2
12.	Dithane M-45	250	0.01	1
13.	Dodemorph	500	0.006	0.6
14.	Plantvax	700	0.004	0.4
15.	SN 513	1,000	0.003	0.3
16.	Nurelle	2,000	0.002	0.2
17.	Delan	2,000	0.002	0.2
18.	Dowco 269	–	0	0
19.	Agrimycin 17	–	0	0
20.	Ferbam	–	0	0

TABLE II

Effects of trunk-injection of some screened fungicides on foliar symptoms in large elm trees after one week

Fungicide	Concentration (ppm)	Symptoms
Vancide TH	1,000	No injury, leaves normal & green.
	5,000	Yellowing of leaves, some injury.
Vancide 40	1,000	Yellowing of leaves, some injury.
	5,000	Browning of leaves, acute phytotoxicity.
SN 513	1,000	Yellowing of leaves, some injury.
	5,000	Acute phytotoxicity.
Validamycin	1,000	No effect.
	5,000	Browning of leaves, phytotoxicity.
Dowicide - A	1,000	No effect.
	5,000	No effect.
Terraclor Super X	1,000	No effect.
	5,000	No effect.

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 at 6 day period was computed. Judging from the ED_{50}, the order of relative toxicity was as follows:-

Lignasan-HCl = Busan 70 > Busan 30-I > BAS 3460 F > Demosan > Orthocide > Dowco 263 > Phaltan > Isobac 20 > Dyrene > Terraclor Super X > Dithane M-45 > Dodemorph > Plantvax > SN 513 > Nurelle = Delan > Dowco 269 = Agrimycin 17 = Ferbam.

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 6 screened fungicides under field conditions showed that only one compound translocated and the rest were either ineffective or phytotoxic to the foliage.

ACKNOWLEDGEMENTS

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REFERENCES

BIEHN, W.L. and A.W. DIMOND (1971). Prophylactic action of benomyl against Dutch elm disease. Phytopathology 55: 179-183.

BERGER, R.D. (1973). Disease progress of *Cercospora abii* resistant to benomyl. Plant Disease Reporter 57: 837.

CLARKE, E.M., P.A. BACKMAN and R. RODRIQUES - KABAME (1974). Cercospora and cercosporidium tolerance to benomyl and related fungicides in Alabama peanut fields. Phytopathology 64: 1476.

DELP, C.J. and H.L. KLOPPING (1968). Performance attributes of a new fungicide and mite ovicide candidate. Plant Disease Reporter 52: 95-99.

LITTEREL, R.H. (1974). Tolerance in *Cercospora arachidicola* to benomyl and related fungicides. Phytopathology 64: 1377.

PRASAD, R. and D. TRAVNICK (1972). Evaluation of fungicide for control of tree diseases. I. Preliminary screening against the Dutch elm disease, *Ceratocystis ulmi* (Buism) Moreau, under laboratory conditions. Dept. of Environment Inf. Rept. CC-X-48.

PRASAD, R. and D. TRAVNICK (1974). Evaluation of fungicides for control of tree diseases. II. Screening against the Dutch elm disease *Ceratocystis ulmi* (Buism) Moreau under laboratory conditions. Dept. of Environment, Report CC-X-75.

APPENDIX A

Hydrogen ion Concentration (pH) of Stock Solutions of Fungitoxic Compounds

	NAME	PHASE	pH
1.	Agrimycin	Powder	5.2
2.	BAS 3460 F	"	9.2
3.	Busan 30-I	Liquid	3.1
4.	Busan 70	"	3.0
5.	Delan 75 WP	Powder	4.8
6.	Demosan 65 WP	"	9.5
7.	Dithane M-45	"	6.5
8.	Dodemorph	Liquid	3.7
9.	Dowco 263	Powder	5.5
10.	Dowco 269	Liquid	4.0
11.	Dyrene	Powder	7.1
12.	Ferbam	Liquid	7.5
13.	Isobac 20	"	7.2
14.	Lignasan-HCl	"	1.0
15.	Nurelle	"	3.2
16.	Orthocide	Powder	8.7
17.	Ortho-Phaltan 50	"	8.7
18.	Plantvax	"	7.3
19.	SN 513	Liquid	5.2
20.	Terraclor Super X	Powder	8.2

APPENDIX B

Chemical Nomenclature of Compounds and their Sources

	COMPOUND	CHEMICAL NAME	SOURCE
1.	Agrimycin 17	Streptomycin Sulfate	Pfizer Co. Limited, Canada
2.	BAS 3460 F	2-Methoxycabamoyl - Benzimidazole	BASF Canada Limited
3.	Busan 30-I	2-(Thiocyanomethylthio) Benzothiazole	Buckman Laboratories Inc., U.S.A.
4.	Busan 70	2-(Thiocyanomethylthio) Benzothiazole	Buckman Laboratories Inc., U.S.A.
5.	Delan 75 WP	5,10-Dihydro-5,10-Dioxonaphtho(2,3,-6)-p-Dithiin-2,3-Dicarbonitrile	Ciba-Geigy Canada Limited
6.	Demosan 65 WP	1,4-Dichloro-2,5-Dimethoxybenzene	DuPont Limited, Canada
7.	Dithane M-45	Complex of Zinc and Maneb (20% Manganese & 2.5% Zinc)	Rohm & Haas Co. of Canada Limited
8.	Dodemorph	N-Cyclododecyl-2,6-Dimethyl-morpholine acetate	BASF Canada Limited
9.	Dowco 263	3,4,5-Trichloro-Pyridine Dicarbonitrile	Dow Chemical, Canada
10.	Dowco 269	[2-Chloro-6-Methoxy-4-(Trichloromethyl) Pyridine]	Dow Chemical, Canada
11.	Dyrene	4,6-Dichloro-N-(2-Chlorophenyl)-1,3,5-Triazin-2-Amine	Chemagro Limited, Canada
12.	Ferbam FC	Ferric Dimethyldithiocarbamate	Chipman Chemicals Limited, Canada

APPENDIX B Cont'd

	COMPOUND	CHEMICAL NAME	SOURCE
13.	Isobac 20	Mono-sodium Salt of 2,2'-Methylene bis (3,4,6-Trichlorophenol)	Kalo Laboratories Inc, U.S.A.
14.	Lignasan-HCl	Methyl 2-Benzimidazole Carbamate Hydrochloride	DuPont Limited, Canada
15.	Nurelle	Methyl 2-Benzimidazole Carbamate Hydrochloride	Dow Chemical, Canada
16.	Orthocide	N-Trichloromethylthio-4-Cyclohexene-1,2-Dicarboximide	Chevron Chemical Limited, Canada
17.	Ortho-Phaltan 50	N-Trichloromethylthio Phthalimide	Chevron Chemical Limited, Canada
18.	Plantvax	5,6-Dihydro-2-Methyl-1,4-Oxathiin-3-Carboxanilide-4,4-Dioxide	Uniroyal Chemical Limited, Canada
19.	SN 513	9-Aza-1,17-Diguanidino-Heptadecane Triacetate	Nor-Am Agr. Prod. Inc., U.S.A.
20.	Terraclor Super X	Pentachloronitrobenzene & Terrazole	Olin Agr. Div., U.S.A.