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Control of Dutch elm disease by the sterol biosynthesis inhibitors fenpropimorph and fenpropidin

R.J. SCHEFFER[1], A.C. BRAKENHOFF[2], A. KERKENAAR[2] and D.M. ELGERSMA[1]

[1] Willie Commelin Scholten Phytopathological Laboratory, Javalaan 20, 3742 CP Baarn, the Netherlands
[2] Institute of Applied Chemistry of the Netherlands Organization for Applied Scientific Research TNO, P.O. Box 108, 3700 AC Zeist, the Netherlands

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Abstract

Sterol biosynthesis inhibitors that inhibit the yeast-hyphae conversion in *Ophiostoma ulmi* suppressed Dutch elm disease development in two elm clones. After curative treatment with fenpropimorph-sulphate of 27 'Vegeta' elms which had previously been inoculated with *O. ulmi*, 25 trees did not show disease symptoms by the end of the second season. All 41 control trees, inoculated with *O. ulmi* only, were clearly diseased. In an experiment with 'Commelin' elms three fenpropimorph salts and thiabendazole were compared. Injection of the trees three weeks after inoculation with *O. ulmi* gave by the end of the second season no symptoms of Dutch elm disease in any of the trees injected with fenpropimorph-phosphate or thiabendazole, and in most trees injected with fenpropimorph-acetate or -sulphate. Similar treatments with the free base of fenpropimorph and fenpropidin-sulphate were less effective due to insufficient uptake of the fenpropimorph emulsion and phytotoxicity of fenpropidin-sulphate, respectively. Injection of fenpropimorph-sulphate or thiabendazole six weeks after inoculation with *O. ulmi* did not result in significant differences from the control group inoculated with *O. ulmi* only.

Fenpropimorph-phosphate and -sulphate completely suppressed Dutch elm disease upon injection of only 7.5 or 10 g per tree (average tree diameter 28 cm). Residue analyses showed only a slow decrease in concentration of the fungicide over two growing seasons and an apparent transport into the new annual ring, other prerequisites for a possible future use for control of Dutch elm disease.

Additional keywords: Ophiostoma ulmi, thiabendazole.

Introduction

The use of sterol biosynthesis inhibitors in agriculture and medicine is rapidly increasing due to their high efficacy and often low toxicity to the host. They are supposed to inhibit fungal growth by blocking specific sites in the sterol biosynthesis pathway by either inhibition of sterol C-14 demethylation or inhibition of the reduction of the C14/15 double bond as well as Δ8-Δ7 isomerization (Lyr, 1987).

Accumulation of ergosterol precursors as a consequence of the sterol biosynthesis inhibition appears to be associated with an irregular deposition of chitin in the cell wall of the fungus, as has been demonstrated for *Candida albicans*, *Ustilago maydis*, *Penicillium italicum*, *Aspergillus fumigatus* and *A. niger* (Barug et al., 1983; Kerkenaar

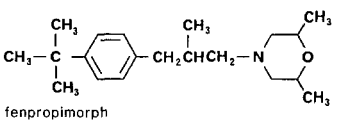
and Barug, 1984; Kerkenaar et al., 1984; Marichal et al., 1984; Hector and Braun, 1987). The existence of this correlation is sustained by the fact that sterols may interact with the activity of chitin synthetase, which is responsible for the formation of chitin (Kerkenaar and Barug, 1984). In budding fungi, chitin is an important factor in septum formation (Cabib et al., 1979, 1982). Irregular deposition of chitin will disturb the normal sequence of cell separation, resulting in chains and clusters of interconnected cells. In most filamentous fungi chitin plays an important role in septum formation as well as in syntheis of the primary cell wall (Trinci, 1978; Burnett, 1979; Aronson, 1981). Irregular deposition of chitin in hyphae may be lethal, causing cells to burst (Kerkenaar and Barug, 1984).

Chitin most probably plays an important role in the dimorphic development of *C. albicans* (Chattaway et al., 1968; Simonetti et al., 1974; Chiew et al., 1980; Mattia et al., 1982). Several authors have reported that sterol biosynthesis inhibitors inhibit its yeast-hyphae conversion (Barug et al., 1983; Borgers et al., 1979, 1980; Borgers, 1980; Haller, 1980; Davies and Marriott, 1981). The irregular deposition of chitin, caused by these sterol biosynthesis inhibitors, may explain the inability of the fungus to grow in the more pathogenic mycelial form.

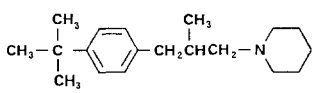
The Dutch elm disease pathogen, *Ophiostoma ulmi* (Buisman) Nannf., has a yeast and a mycelial form and therefore can be referred to as a dimorphic fungus. This dimorphism may be essential in pathogenesis. Budding cells are passively transported by the transpiration stream within the individual xylem elements (Banfield, 1968; Campana, 1978). However, it is very unlikely that spread of the fungus from one vessel to another can be accomplished by these cells, since pit membranes have to be penetrated. In several studies penetration of vessel pits was observed for hyphae only (Miller and Elgersma, 1976; Elgersma and Steerenberg, 1978; Scheffer and Elgersma, 1982). As extensive spreading of the fungus throughout the vascular system is necessary for disease development, inhibition of the conversion from the yeast to the mycelial phase by sterol biosynthesis inhibitors may prevent development of symptoms of Dutch elm disease.

It was shown that the yeast – hyphae conversion in *O. ulmi* can be inhibited in vitro by various sterol biosynthesis inhibitors (Kerkenaar et al., 1986, and unpublished). Fenpropimorph was the most effective one of the compounds tested, inhibiting mycelial growth entirely at a concentration of 50 $\mu g\ l^{-1}$.

In this study the efficacy of fenpropimorph and of several related compounds (Fig. 1) in controlling Dutch elm disease after trunk injection was tested. Fenpropimorph concentrations within the trees were monitored for two seasons in elms that were carefully observed for signs of Dutch elm disease or phytotoxicity.



fenpropimorph



fenpropidin

Fig. 1. Basic chemical structure of the sterol biosynthesis inhibitors used in this study.

Materials and methods

Plant material and trial setup. Experiments were carried out in Flevoland with the elm clones *Ulmus* × *hollandica* 'Vegeta' and 'Commelin', both being susceptible to the aggressive strain of *O. ulmi*. A plot of 12-year-old 'Vegeta' elms was used for the first experiment which was carried out in 1985-1986; 14-year-old 'Commelin' elms were used in the second experiment, in 1986-1987. Both groups of trees were in the same diameter range, averaging 26 cm diameter at breast height (DBH).

All trees in one plot were numbered and thereafter grouped at random.

Pathogen and inoculation. the isolates H6 and H106 of the aggressive strain (North American race; cf. Brasier, 1984) were grown as shake cultures for 4-7 days at 23 °C in Tchernoff's medium (Tchernoff, 1965). Conidial suspensions were prepared by filtering the cultures through glasswool in order to remove mycelium. Subsequently the conidia were washed three times with sterile tap water by centrifugation (10 000 *g* for 10 min). The final suspensions were adjusted to 5×10^6 conidia per ml. Equal volumes of the suspensions of H6 and H106 were mixed and used for inoculum.

For inoculating elms, 50 μl of the inoculum was placed on the tip of a Stanley trim knife; then a wound was made in a branch allowing the suspension to be sucked in. Three branches per tree, at an average height of approx. 7 to 8 m, were inoculated twice on opposite sides for the first experiment, whilst in the second experiment only one branch was inoculated, also twice on opposite sides. All inoculations were done on June 6 and 7, 1985 for the first experiment and on June 2-4, 1986 for the second experiment.

Fungicides, dosage and application. The following fungicides were used in the field trials: fenpropimorph free base (emulsifiable concentrate with 750 g active ingredient (a.i.) l^{-1}), fenpropimorph-sulphate (200 or 250 g a.i. l^{-1}), fenpropimorph-phosphate (200 g a.i. l^{-1}), fenpropimorph-acetate (200 g a.i. l^{-1}), fenpropidin-sulphate (250 g a.i. l^{-1}) and, for reference purposes, thiabendazole (220 g a.i. l^{-1}). All fungicides except thiabendazole came from Dr R. Maag AG, Dielsdorf, Switzerland. Thiabendazole formulated for control of Dutch elm disease (trade name Lirotect Ulmi 20S) was purchased from Ligtermoet Chemie BV (Roosendaal, the Netherlands).

Fenpropimorph and fenpropidin derivatives were injected into the trees in a standard volume of deionized water: 1 l in the first experiment and 3 l in the second. Thiabendazole (1.833 g cm^{-1} tree DBH) was injected in a standard volume of 20 l tap water.

Low pressure injection was used (Gkinis and Stennis, 1980). Hand-pumped pressure tanks with a volume of 10 l (type 172T, Gloria Werke, Wadersloh, BRD) were equipped with an injection harness made of 13 nylon injection tees (Elm Research Institute, Harrisville, NH) connected with 25 cm of silicon rubber tubing. Holes of 8 mm in diameter were drilled 4 cm deep into the tree using a battery-powered drill. They were drilled c. 8 cm apart and c. 10 cm above ground level. Immediately after drilling the holes, the injection tees were inserted by hand. No excessive force was used as this could cause the sapwood to be compressed resulting in an unsatisfactory uptake of the fungicide. Before inserting the last tee the harness was filled with the fungicide. A pressure of 100 to 150 kPa (15 to 22 psi) was used for injection. After injection the holes were left open.

Disease ratings. Evaluation of disease severity was based on an arbitrary disease index defined as follows (modified after Scheffer, 1983):

0: healthy
1: flaccidity or yellowing of some leaves
2: wilting, yellowing or partial necrosis of some leaves; tree clearly affected, but disease restricted to inoculated branches
3: wilting, yellowing or partial necrosis of many leaves; symptoms also in non-inoculated twigs or branches
4: severe wilting and necrosis of most leaves, more than one shoot or tip of a branch dying and crooked but still some non-affected twigs or branches present
5: leaves fallen and/or brown, shoots and tips of branches dead and crooked.

Fungicide recovery from elm wood. To determine transport and persistence of the fungicides in the elms, branches from trees treated with fungicide or from control trees were sampled 3, 10 and 15 months after inoculation with the pathogen. The branches were sealed in polyethylene bags and stored at -80 °C until analysis.

After removing the bark, samples of 5 g of wood were clipped into pieces and homogenized in 50 ml of 1.8 M KOH in methanol for 5 min using a Bühler homogenizer. The slurry was kept at room temperature for 16-24 h to saponify and then extracted twice with an equal volume of hexane. The hexane fraction was dried with a rotary vacuum evaporator and the residue redissolved in a few ml of hexane. After centrifugation (1400 *g* for 5 min) the supernatant was transferred to a small vial. One ml of hexane containing 0.1 mg methyl stearate (Merck) was added as an internal standard. The mixture was evaporated to dryness under nitrogen, redissolved in 100 μl of hexane and used for gas chromatography.

Quantitative fungicide analysis. A Packard 430 gas chromatograph with a flame ionization detector was routinely used. All separations were performed on a CP Sil 5B (26 m $\times$ 0.22 mm) fused silica WCOT capillary column. Column flow was 0.3 ml N_2 min^{-1}; split flow was 40 ml N_2 min^{-1}. Injection port and detector temperature were 250 °C. The column oven temperature was programmed from 180 °C till 210 °C at a rate of 3 °C min^{-1} and from 210 °C til 280 °C at a rate of 10 °C min^{-1}. One μl of sample was injected. The same column and comparable flow and temperature conditions were used for control experiments in which a mass spectrometer was used for detection. The instrument used was a Hewlett-Packard 5993 B gas chromatograph – mass spectrometer. The recovery of the fungicide from elm wood, based on known amounts of fungicide added to pieces of debarked branches before homogenizing, was 61 $\pm$ 3 %. This recovery factor of 61 % was used for the calculation of all data presented.

Results

Under Dutch weather conditions very few of even the most susceptible elms die in the year they are inoculated with *O. ulmi*. In the present experiments, Dutch elm disease progressed steadily in the control trees, i.e. those without fungicide, (Table 1 and 2), resulting in 68 % dead and dying trees by the end of the second season in both the experiments with 'Vegeta' and with 'Commelin' elms (Fig. 2). In the experiment with 'Vegeta' elms, only one of the elms that were injected curatively with various doses of

Table 1. Average disease indices of 'Vegeta' elms after inoculation with *O. ulmi* on June 6, 1985 followed by injection with sterol biosynthesis inhibitors. Averages were calculated for the fungicide treatments because no dose response relations were found.

Fungicide treatment (g/tree)	Number of trees	Weeks after inoculation	Average disease indices				
			1985 July 23	1985 Aug. 21	1985 Sep. 12	1986 July 9	1986 Aug. 13
Control (no fungicide)	41	—	2.5	3.0	3.2	3.9	4.4
Fenpropimorph-sulphate							
7.5	6	2	1.8	1.3	1.6	0.2	0.2
22.5	6	2	1.8	1.7	2.1	1.3	1.0
30	10	3	2.4	1.9	2.1	1.4	1.4
75	5	3	2.0	1.9	2.0	0.6	1.2
Totals	27		2.0**	1.7**	1.9**	0.9**	1.0**
Fenpropimorph (free base)							
2.25	6	2	2.1	2.2	2.8	2.6	3.5
7.5	6	2	2.1	2.2	2.8	2.6	3.5
22.5	6	1	1.3	1.3	1.2	1.4	1.6
22.5	3	2	2.2	2.2	2.5	1.2	1.3
22.5	5	4	2.4	2.3	2.5	2.1	2.9
22.5	12	5	2.5	2.4	2.5	2.3	2.5
225	6	4	2.5	2.2	2.0	3.1	3.0
Totals	44		2.2*	2.1**	2.2**	2.2**	2.6**
Fenpropidin-sulphate							
7.5	6	2	1.5	1.8	2.0	0.5	0.7
22.5	7	2	1.9	1.8	2.5	0.6	0.9
75	6	3	2.8	2.4	2.9	2.0	1.7
Totals	19		2.1*	2.0**	2.5**	1.0**	1.1**

*, **: Disease index significantly different from that of the control trees at that date at $p < 0.01$, < 0.001, respectively (Wilcoxon two sample test, two-tailed).

fenpropimorph-sulphate solution died, one showed local symptoms (disease index 2), whilst the others did not show recognizable Dutch elm disease symptoms (Fig. 2). Results with the free base of fenpropimorph were variable. Finally, only 36 % of the elms injected with the free base of fenpropimorph did not show Dutch elm disease symptoms. A clear correlation between fenpropimorph (free base) dose and efficacy or phytotoxicity could not be assessed. In the second experiment, with 'Commelin' elms, we tested several fenpropimorph salts and thiabendazole, the only fungicide currently on the market for control of Dutch elm disease. All fungicides that were applied three weeks after inoculation with *O. ulmi*, when disease symptoms were already clearly present, resulted in control of Dutch elm disease. Injection of fenpropimorph-sulphate or

Table 2. Average disease indices of 'Commelin' elms after inoculation with *O. ulmi* on June 2-4, 1986 followed by injection with sterol biosynthesis inhibitors or thiabendazole.

Fungicide treatment (g/tree)	Number of trees	Weeks after inoculation	Average disease indices		
			1986 Aug. 14	1987 Aug. 21	1987 Sep. 11
Control (no fungicide)	22	—	1.8	3.8	4.0
Fenpropimorph-acetate 10	10	3	1.5	0.6**	0.9**
Fenpropimorph-phosphate 10	9	3	1.1*	0.2**	0.4**
Fenpropimorph-sulphate 10	10	3	0.8*	0.6**	0.8**
10	10	6	2.4*	2.7	3.1
Thiabendazole 126-165[1]	10	3	1.3	0.1**	0.1**
132-150[1]	9	6	3.3*	3.9	3.9

*, **: Disease index significantly different from that of the control trees at that date at $p < 0.01$, < 0.001, respectively (Wilcoxon two sample test, two-tailed).
[1] Amount injected was 1.833 g cm^{-1} tree circumference (measured at brest height).

thiabendazole six weeks after inoculation with *O. ulmi* did not result in significant differences from the control group inoculated with *O. ulmi* only (Table 2 and Fig. 2).

Phytotoxicity was noticed in fenpropimorph-sulphate treated trees after the winter, especially at higher dosages (Table 3). A limited concentration series showed a straightforward correlation between dosage and phytotoxicity. Fenpropidin-sulphate, although it seemed to suppress development of Dutch elm disease symptoms effectively (Table 1 and Fig. 2), caused severe phytotoxic effects at the concentrations used (Table 3). In the second experiment, the effect of fenpropimorph-acetate, -phosphate and -sulphate was compared. Injection with the acetate resulted in variable reactions from the trees and phosphate appeared to be less toxic than sulphate.

The amounts of fenpropimorph or fenpropimorph-sulphate recovered from branches varied, not only from tree tot tree, but also from branch to branch within one tree (Table 4 and 5). The recovered amounts of the free base of fenpropimorph from branches of fenpropimorph-injected trees were averaging only 3 to 6 % of that of fenpropimorph-sulphate after injection of an equal amount of active ingredient (Table 4). The fungicide was apparently transported into the new annual ring of the tree; the total amount recovered from the annual rings of 1985 (the year of injection) plus 1984 plus 1986 (the outermost ring) equalled the amount recovered from a subsequent total branch sample (Table 6).

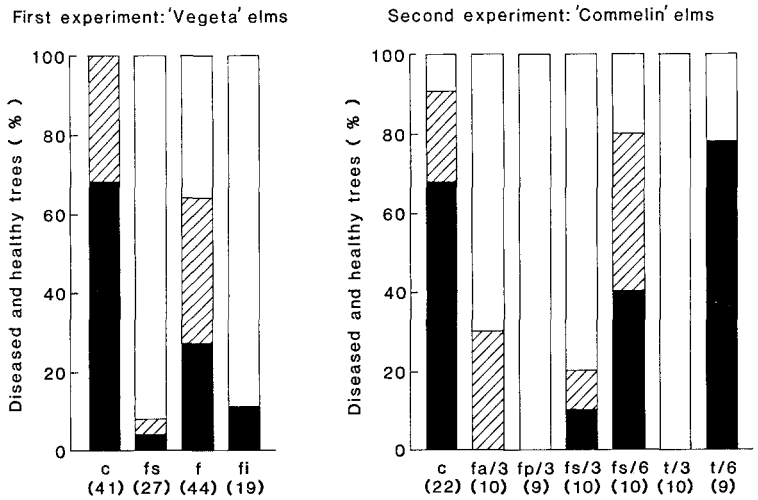


Fig. 2. Comparison of the major effects of the fungicide treatments on Dutch elm disease symptom development by the end of the second season (cf. Table 1 and 2). Unshaded is the percentage of trees not showing symptoms of Dutch elm disease (disease index less than 2). Black is the percentage of dying and dead trees (disease index over 4). The shaded area then represents the percentage of trees not (yet) dying but clearly showing symptoms. Between brackets the number of trees per treatment.

Abbreviations: c: control (*O. ulmi* only); fs: fenpropimorph-sulphate; f: fenpropimorph (free base); fi: fenpropidin-sulphate; fa/3: fenpropimorph-acetate, 3 weeks after *O. ulmi*; fp/3: fenpropimorph-posphate, 3 weeks after *O. ulmi*; fs/3: fenpropimorph-sulphate, 3 weeks after *O. ulmi*; fs/6: fenpropimorph-sulphate, six weeks after *O. ulmi*; t/3: thiabendazole, three weeks after *O. ulmi*; t/6: thiabendazole, six weeks after *O. ulmi*.

Discussion

Fenpropimorph derivatives, such as the sulphate, phosphate and acetate used in the experiments described here, apparently did suppress Dutch elm disease effectively. Recurrence of Dutch elm disease in the second year was rare in the trees injected with one of the fenpropimorph salts. The exception were those trees that had been treated six weeks after inoculation with *O. ulmi*: injection of neither fenpropimorph-sulphate nor thiabendazole arrested symptom development in the diseased trees. These observations confirm the claim that fungicide treatments are only useful in the initial phase of the disease, ususally quantified as 5 to 10 % crown damage at a maximum (Greig and Coxwell, 1983).

Injection with the free base of fenpropimorph did generally not prevent Dutch elm disease recurrence. However, the free base of fenpropimorph was developed for foliar spray application and the formulated product proved not suitable for injection into elms. Injection of the emulsion proceeded with a steady decrease of flow, apparently because of blockage of the xylem. The relatively low phytotoxicity observed for the free base of fenpropimorph was probably due to poor uptake and transportation in the elms, an assumption supported by the low recovery of the active compound from branches (Table 4). Fenpropidin-sulphate, on the other hand, caused such severe

Table 3. Numbers of elms per phytotoxicity class one year after inoculation with *O. ulmi* followed by injection of sterol biosynthesis inhibitors or thiabendazole. The total number of trees is not always identical to the number within one treatment as presented in Tables 1 and 2, as for some badly affected trees phytotoxicity ratings were not possible.

Fungicide treatment (g/tree)	Weeks after inoculation	Phytotoxicity class[1]				
		none	slight	serious	severe	extreme
'Vegeta' elms						
Control (no treatment)	—	24				
Fenpropimorph-sulphate						
7.5 g/tree	2		4	2		
22.5 g/tree	2			1	5	
30 g/tree	3		3	3	4	
75 g/tree	3	1	1	1	2	
Fenpropimorph (free base)						
2.25 g/tree	2	4	2			
7.5 g/tree	2	5	1			
22.5 g/tree	2-5	11	10	4		1
225 g/tree	4	4	2			
Fenpropidin-sulphate						
7.5 g/tree	2			3	2	
22.5 g/tree	2			1	6	
75 g/tree	3				3	3
'Commelin' elms						
Control (*O. ulmi* only)	—	22				
Fenpropimorph-acetate						
10 g/tree	3	5	2	1	2	
Fenpropimorph-phosphate						
10 g/tree	3	2	4	3		
Fenpropimorph-sulphate						
10 g/tree	3		2	7		
0.5 g/tree (no *O. ulmi*)		2				
2 g/tree (no *O. ulmi*)		2				
10 g/tree (no *O. ulmi*)			1		1	
30 g/tree (no *O. ulmi*)					2	
Thiabendazole						
126-165 g/tree[2]	3	8	2			

[1] Phytotoxicity was classified as follows: none – no symptoms; slight – locally some dead twigs; serious – many dead twigs; severe – dieback of branches; extreme – dead tree.
[2] At a rate of 1.833 g cm^{-1} tree circumference (measured at breast height).

Table 4. Recovery of fenpropimorph or its sulphate from 'Vegeta' elms. At every date five treated and three control trees were sampled. Data given are averages from two or three branches per tree.

Treatment	Concentration ($\mu g\ g^{-1}$ wood)		
	1985 Aug. 27	1986 April 28	1986 Aug. 26
Fenpropimorph-sulphate			
22.5 g/tree	48	64	45
	24	29	23
	9.4	8.0	4.2
	5.1	6.3	1.9
	3.4	3.5	0.6
Average	18	22	15
7.5 g/tree			6.8
			4.3
			1.4
			1.1
			0.4
Average			2.8
Fenpropimorph (free base)			
22.5 g/tree	1.6	1.8	0.6
	1.5	1.2	0.5
	1.0	0.7	0.5
	0.8	0.6	0.3
	0.4	0.6	0.0
Average	1.1	1.0	0.4
Controls	0.3[1]	0.2[1]	0.0
	0.3[1]	0.1[1]	0.0
	0.0	0.0	0.0

[1] Low concentration of fenpropimorph detected in one sample per series only. Concentations detected were between 0.22 and 0.47 $\mu g\ g^{-1}$ wood and are thought to be carry-over contamination of glassware. Controls were invariably nil in the last series in which special care was taken to avoid such contamination.

phytotoxic effects that no conclusion on its effect on Dutch elm disease symptom development could be drawn: dieback of branches interfered with normal disease development.

Both fenpropimorph and its sulphate could readily be recovered from branches and apparently disappeared only slowly. Transport into the new annual ring could be demonstrated by assessing the concentration in separate annual rings. The amount of fenpropimorph-sulphate recovered from a 5 g sample of a seven-year-old branch was the same as that from the combined rings of 1984, 1985 (the year of treatment) and

Table 5. Recovery of fenpropimorph-sulphate from 12 branches per 'Vegeta' elm sampled from 7-8 m height 14 months after injection of 30 g into the trunks of the trees.

Tree nr.	Average concentration ($\mu g\ g^{-1}$ wood)	Minimum and maximum concentration determined ($\mu g\ g^{-1}$ wood)
84	42	5.1- 68
86	45	0.6-107
88	23	5.1- 75

Table 6. Distribution of fenpropimorph-sulphate within one seven-year-old branch, 14 months after injection of 22.5 g into the trunk of the tree.

Part of branch	Growth ring weight as percentage of total branch weight[1]	Concentration ($\mu g\ g^{-1}$ wood)	Concentration as calculated for a total branch sample[2] ($\mu g\ g^{-1}$)
Growth ring 1984	19	40	19/100 × 40 = 7.6
Growth ring 1985	13	89	13/100 × 89 = 11.6
Growth ring 1986	6	61	6/100 × 61 = 3.7
Total	38		22.9
Total branch sample[2]	100	23	23

[1] The growth rings of 1984 + 1985 + 1986 comprised 38 % of the sample weight (i.e. in September 1986 62 % was wood from 1983 and older).
[2] Including growth rings before 1984; exclusieve bark.

1986 from a similar sample of the same branch. Apparently all of the fenpropimorph-sulphate was retained in these three growth rings. Fourteen months after injection of 7.5 g fenpropimorph-sulphate the average concentration in branches was still 2.8 $\mu g\ g^{-1}$ wood (Table 4); most probably well above the concentration needed to prevent mycelial growth of *O. ulmi* (0.05 $\mu g\ ml^{-1}$ in vitro). One tree injected with 7.5 g fenpropimorph-sulphate from an experiment in the city of Amsterdam (data not shown) was sampled in the third season, 26 months after application. No phytotoxicity was present, the concentration of fenpropimorph-sulphate was 0.62 ± 0.49 $\mu g\ g^{-1}$ wood, again showing that the fungicide disappears only slowly.

Phytotoxicity was not observed in the year of treatment, but in the second year some, in our opinion at a tolerable level, phytotoxicity was apparent in the trees that received 7.5 g of fenpropimorph-sulphate. No phytotoxicity was observed in the trees that had received less, and more in the trees that had received higher dosages. A comparison of the sulphate, acetate and phosphate salts leads to the conclusion that especially the phosphate may be attractive for further research. No recurrence of Dutch elm disease was observed in the second year and phytotoxicity was lower than that of the other salts. Generally, phytotoxicity symptoms resembled frost damage, which, although the

winters of 1985 and 1986 were unusually severe, was not present in any of the control trees. It was hypothesized that frost hardiness is related to the composition of the cell membrane (Uemura and Yoshida, 1984). Probably the fungicide treatments resulted in alterations in the sterol pattern of the trees' cells, which then ultimately may have resulted in a decreased frost hardiness.

A practical therapeutic control for Dutch elm disease has to be effective in suppression of the disease for a reasonable price per tree. The most common fungicide used for therapeutic control of Dutch elm disease is thiabendazole. In this study its effectiveness to control Dutch elm disease is not questioned, as long as it is used in the initial phase of the disease. In contrast to common procedures and for practical purpose, we injected the desired amount in only 20 liters into the trees, instead of the 41 to 55 liters recommended (depending on tree diameter; Gkinis and Stennes, 1980), without noticing adverse effects if applied three weeks after inoculation with *O. ulmi*. Of course it cannot entirely be ruled out that injection of thiabendazole in a larger volume six weeks after *O. ulmi* would have resulted in some effect. Two major practical differences with the sterol biosynthesis inhibitors used for this study are the amount of active ingredient used per tree and the total volume to be injected. It is usually recommended to inject into a 26-cm diameter elm (DBH) as used for this study 148 g thiabendazole in 49 liters (Gkinis and Stennis, 1980). For the sterol biosynthesis inhibitors only an amount of one or three liter was injected per tree, which is promising from a practical (and economical) point of view. Suppression of Dutch elm disease by the fenpropimorph salts tested was such that for further research an amount of one gram of active ingredient per 10 cm tree circumference at breast height (identical to 0.3 g cm^{-1} DBH) should be the maximum to be tested. The complete suppression of Dutch elm disease observed after injection of 7.5 g of fenpropimorph-sulphate (in 1 liter), the apparent transport of the compound throughout the tree and into the new annual ring and the slow decrease in concentration with time suggest the usefulness of fenpropimorph salts for control of Dutch elm disease.

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Samenvatting

Bestrijding van de iepeziekte met de sterolbiosyntheseremmers fenpropimorf en fenpropidin

De iepeziekte kan onderdrukt worden door sterolbiosyntheseremmers die de overgang van *Ophiostoma ulmi* van de gistvorm in de hyfevorm remmen. Aan het eind van het tweede seizoen na een curatieve behandeling van 27 'Vegeta' iepen met fenpropimorfsulfaat bleken 25 bomen geen symptomen van iepeziekte te vertonen. Alle controlebomen, die alleen met *O. ulmi* geïnoculeerd waren, waren duidelijk ziek. In een proef met 'Commelin' iepen werden drie fenpropimorfzouten en thiabendazool vergeleken. De

zouten werden drie weken na de inoculatie met *O. ulmi* geïnjecteerd. Aan het eind van het tweede seizoen vertoonden geen van de bomen die met fenpropimorffosfaat of thiabendazool geïnjecteerd waren en slechts enkele bomen die met fenpropimorfacetaat of -sulfaat geïnjecteerd waren iepeziektesymptomen. Behandelingen met fenpropimorf (vrije base) en fenpropidinsulfaat werkten minder goed door de slechte opname van de fenpropimorfemulsie en de fytotoxiciteit van fenpropidin. Injectie met fenpropimorfsulfaat of thiabendazool zes weken na inoculatie leidde niet tot significante verschillen met de controlegroep die alleen met *O. ulmi* geïnoculeerd was.

Een dosis fenpropimorffosfaat of -sulfaat van 7.5 of 10 g per boom met een gemiddelde boomdiameter van 26 cm bleek de iepeziekte volledig te kunnen onderdrukken. Uit residue-onderzoek bleek dat de concentratie van het fungicide gedurende de twee groeiseizoenen slechts langzaam afnam en dat het middel naar de nieuwe jaarring werd getransporteerd, twee voorwaarden voor een toepassing op praktijkschaal van fenpropimorf voor de bestrijding van de iepeziekte.

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