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FUNGICIDES FOR DUTCH ELM DISEASE: COMPARATIVE EVALUATION OF COMMERCIAL PRODUCTS

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Abstract. The commercial fungicide formulations, Arbotect 20-S, Phyton-27, Fungi-Sol, and biological product, Binab-T, were injected into juvenile (ave. 17.5 cm diameter) American elms prior to (prophylactic) or after (therapeutic) the trees were inoculated with the Dutch elm disease (DED)-causing fungus, *Ceratocystis ulmi*. Samples of all trees were cultured to verify establishment of *C. ulmi* and DED symptom development was monitored for two growing seasons. At the end of the second growing season none of 20 Arbotect-injected trees (prophylactic and therapeutic) displayed DED symptoms. Fungi-Sol and Binab prophylactic groups (5 and 10 trees, respectively) contained significantly fewer diseased trees than the control group, but no treatment, other than Arbotect, showed significant therapeutic effect. Sapwood damage associated with each of the treatments was greater than that associated with injection of water. Discolored sapwood was localized around injection sites in Fungi-Sol and Binab-treated trees, but extended a maximum of 2 m and 6 m, respectively, above the points of injection of Arbotect and Phyton. Injection holes were overgrown and closed for all water, Fungi-Sol, and Binab treatments while one Arbotect and five Phyton-treated elms had injection holes that remained open and were weeping with bacterial slime flux. Two trees treated with a high dosage of Phyton had an aggregate of five open lesions associated with injection holes.

Résumé. Les fongicides commerciaux, l'Arbotect 20-S, le Phyton-27, le Fongi-Sol et un produit biologique, le BINAB-T, furent injectés dan des jeunes ormes d'amérique (moyenne de 17.5 cm de diamètre) avant (prophylactique) ou après (thérapeutique) que les arbres furent inoculés avec le champignon causant la maladie hollandaise de l'orme (MHO), *Ceratocystis ulmi*. Des échantillons de tous les arbres furent cultivés afin de vérifier l'établissement du *C. ulmi*, et le développement des symptômes de la MHO fut observé pendant deux saisons de croissance. A la fin de la deuxième saison de croissance, aucun des arbres traités à l'Arbotect 20-S (traitement prophylactique ou thérapeutique) ne présentait des symptômes de la MHO. Les groupes "prophylactiques" traités au Fongi-Sol et au BINAB (5 et 10 arbres, respectivement) présentaient significativement moins d'arbres malades que le groupe contrôle, mais aucun traitement autre qu' à l'Arbotect, ne présentait un effet thérapeutique significatif. Les dommages à l'aubier associés à chaque traitement furent plus élevés que ceux associés à l'injection d'eau. Une zone décolorée était localisée autour des sites d'injection des arbres traités au Fongi-Sol et au BINAB, mais s'étendait jusqu'à un maximum de 2 m à 6 m, respectivement, *au-dessus des points d'injection à l'Arbotect et au Phyton. Les trous* d'injection étaient refermés sur les arbres traités à l'eau, au Fongi-Sol et au BINAB, tandis qu'un arbre traité à l'Arbotect et cinq traités au Phyton présentaient des trous d'injection ouverts et d'où s'écoulait un flux bactérien. Deux arbres traités avec un dosage élevé de Phyton avaient un ensemble de cinq lésions ouvertes associées aux trous d'injection.

Systemic fungicides for Dutch elm disease (DED) control have been commercially available since 1972, but the effectiveness of some of the products remain to be rigorously tested by independent investigators. Another aspect of concern about repeated injection of fungicide to protect elms against DED is wounding (Shigo and Campana 1977). This paper presents results of a two-year experiment in which elm trees treated with Arbotect-20®, Fungi-Sol®, Phyton-27® and Binab-T® (see Table 1) were challenged by inoculation with *Ceratocystis ulmi* (the DED-causing fungus), monitored for DED symptoms, and dissected to ascertain the extent of injection damage.

Benzimidazol compounds used in Arbotect and Lignasan® (= Elmosan®, Elm Fungicide®) have been conclusively demonstrated to suppress *C. ulmi* and symptom development (3, 4, 12). The active molecules in the two products are about equally effective, but the activity of Arbotect persists longer (6, 10, 11). Thus, current label recommendations for Arbotect provide for an injection dosage that will protect an elm for three years while Lignasan label requires annual injections. Andrews et al. (1) showed that the volume of discolored sapwood in elms injected with the 3-year Arbotect dosage was 3.5-times that in elms injected with an annual dosage of Lignasan, but these authors did not comment on the cumulative effect of three annual injections versus

a single treatment in three years.

Fungi-Sol is in the same fungicide class as Lignasan and Arbotect, but I could find no published data on the effectiveness of this product against *C. ulmi in vitro.* Constonis (2) reported that, over a three-year period (1979-1981), there were no new DED infections among 224 elms prophylactically injected with Fungi-Sol as part of an elm management program. Remission of DED symptoms occurred in 22 of 28 diseased elms administered therapeutic injections of Fungi-Sol plus pruning of diseased sections (2). While these results are encouraging, the work lacked experimental controls and did not rigorously demonstrate that Fungi-Sol was a factor in prophylaxis or therapy.

In their promotional literature, producers of Phyton (a form of copper sulphate) present data from limited uncontrolled field trials, but no information on internal damage caused by the product. As is the case with Fungi-Sol, Phyton was registered after 1979 when the Environmental Protection Agency dropped the requirement that a product must be effective in the uses for which it is registered.

Binab was reported to reduce loss rates of elms in one field trial in France (8). The active ingredient in this product is a fungus. *Trichoderma viride* Persoon, which is benign to the tree but antagonistic to many other fungi, including *C. ulmi.* For more than 10 years this fungus has been injected into fruit trees to control silver leaf disease (caused by *Chondrostereum purpureum)* in Europe, but this product has not been registered for use in the United States.

Materials and Methods

Trees used in this study were young American elms (15-30 years old) growing in open to partially closed-canopied stands on abandoned farm land near the village of Bridgeport in Onondaga County, New York. During 1985, a total of 100 elms were selected for treatment. Criteria for selection were:

1. No symptoms of DED in the selected tree or in other elms within 10 m of the selected tree.
2. Dominant or codominant crown position.
3. Uniformity of diameter (9-24.5 cm; mean of 17.5 ± 3.4).
4. Minimum distance of 10 m between selected trees.

Each tree selected was marked with a numbered aluminum tag and circumferencial paint stripe. Treatments were assigned randomly in the lab without reference to plot. Each tree was injected with water only or with one of the materials listed in Table 1.

Rather than being based strictly on diameter, dosage of Phyton (ND) and Arbotect were calculated to approximate the concentration of active ingredient per unit of bark surface area achieved in a 63.5 cm (25") diameter elm tree in-

Table 1. Fungicides, formulations, and dosages used in DED challenge tests.

Commercial name	*Label formula*	*AI in gm/cm diam. label*	*actual*[b]
Fungi-sol[R]	4 gm 2% AI in Mauget caps, 1 cap/2" .diam.	0.016	.021
Binab-T[R]	1 gm dry pellet in drill hole, in sapwood, 1 pellet/10 cm circ.	0.039	.018
Arbotect-20S[®]	12 oz. of 26.6% AI diluted 1:40 in water/5" diam.	7.07[c]	1.79[d]
Phyton-27[R] (ND)	56 gm 21.4% AI concentrate + 1624 ml diluent + 2.5 gal. water/tree	0.19[c]	0.05[d]
Phyton-27 (HD)	" " ditto " "	0.67	0.67[e]

[a]Chemical names and proprietors are as follows: Fungisol, 2-(2-ethoxyethoxy) ethyl-2-benzimidazol carbamate, J.J. Mauget Co. Burbank, California; Binab-T, *Trichoderma viride* (a fungus), Bio-innovation AB, Sweden; Arbotect-20S, 2-(4-thiazolyl) benzimidazol hypophosphite, AGVET (division of Merck and Company), Rahway, New Jersey; Phyton-27, copper sulfate pentahydrate, Source Technology Biologicals, St. Paul, Minnesota. For Phyton, (ND) = normal dosage, (HD) = high dosage.

[b]The mean of actual dosages calculated for tree diameter at 1.4 m height.

[c]Dosage for 25" (63.5 cm) diameter tree.

[d]Dosage for test trees adjusted to be equivalent AI/dm^2 bark surface area in a 25" (63.5 cm) diameter tree treated at the label rate (O'Callaghan et al. 1980).

[e]Mean dosage when the full commercial package was injected in one test tree.

jected as directed on the labels of the respective products. This conversion is necessary to avoid treating the small experimental elms with unrealistically high, and perhaps phytotoxic, concentrations. Although our test elms averaged (17.5 cm), about 1/4 of the diameter of a nominal typical street elm (60.3 cm), they had only 1/10 of the bark surface area. The relationship of bark surface area to diameter derived by O'Callaghan *et al.* (7) is illustrated in Figure 1.

Arbotect and Phyton in tap water mixtures were injected at 1.3 to 2 atmos. (5-15 psi) from plastic 12 L spray tanks rigged with plastic tubing and spiles. Binab pellets were placed in freshly drilled holes plugged with grafting wax. Fungi-Sol was introduced from Mauget® pressurized capsules. Injected materials were delivered in holes (1 hole per 4 cm tree diameter) either 4.4 mm (11/64", Fungi-Sol) or 6.7 mm (17/64", other materials) in diameter drilled about 1.5 cm deep into root flares just above ground level. Drilling in this manner minimized injury to the tree (9).

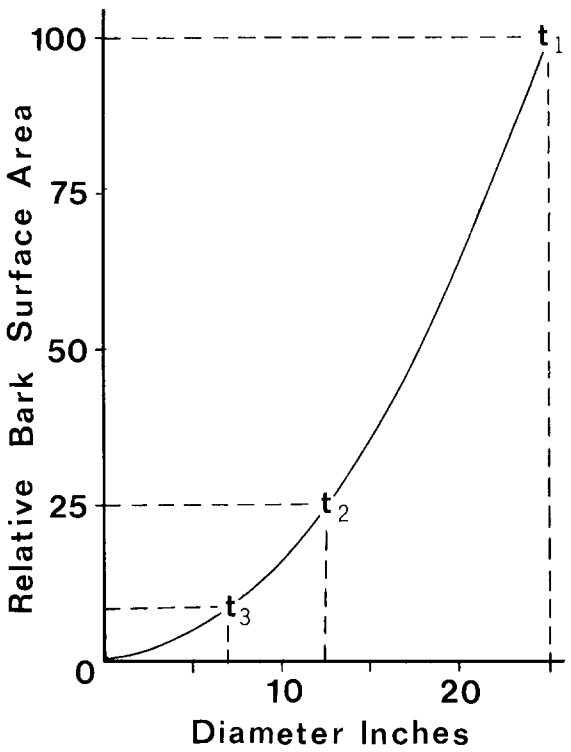

Fig. 1. Relationship between diameter (dbh) and bark surface area (bsa) from the formula log bsa = 0.236 + 1.90 log dbh (O'Callaghan et al., 1980). the "typical" street elm 25 inches in diameter (t_1) has 4 times the bsa of a tree (t_2) 1/2 of its diameter and 10 times that of our average plot tree (t_3), approximately 1/4 its diameter. Thus, basing dosages of systemic chemicals on diameter tends to overdose small trees and under dose trees that are considerably larger than trees for which the dosage was derived.

Fungicide treatments were either prophylactic (before infection) or therapeutic (after infection). Prophylactic treatments of Binab were made April 12, 1985; those with other materials were completed between May 29 and June 30. Therapeutic treatments occurred between August 6 and 28, 1985. Earliest therapeutic treatments were given to trees expressing foliar symptoms of DED. After about August 20 "therapeutic" injections were done without regard to symptom development. No tree was treated more than once or with more than one substance.

From June 26 to July 5 all plot trees were inoculated with spores of *Ceratocystis ulmi* aggressive strains (University of Wisconsin 824 and 70-64) by pipeting 100 µl of spore suspension (in distilled water) into a 2 mm diameter hole drilled in one twig crotch on each of four branches in the lower 1/3 of the tree crown.

Inoculated branches were inspected and sampled during August 1985. Pieces of the samples were cultured 10-30 days on potato dextrose agar at 18°C to test for presence of viable *C. ulmi.* In September 1985 and May, August, and September 1986 trees were inspected for foliar symptoms and sapwood streaking. During September 1986, 4-7 trees given water or each product were dissected. Boles were cut at the root collar (level 0) and at 1 m intervals thereafter. Cross sectional area of discolored sapwood associated with injection wounds was measured at each level. Each section was also inspected for DED-associated streaking in 1985 and 1986 annual rings. No bioassays for fungicidal activity in tissues of treated elms were made.

Results

Recovery of Ceratocystis ulmi in Culture. C. ulmi, was isolated from most of the inoculated branches in all treatments (Table 2, column A). Failure to recover the fungus in 19 experimental trees was probably most often due to the presence of aggressive contaminating saprophytic fungi on or in the twig sampled rather

than failure of the inoculation to establish the fungus. Six of the 19 trees for which samples were negative later developed DED symptoms that apparently spread from inoculated branches. We therefore can conclude that inoculations were successful in at least 87 of 100 elms in the experiment. Differences in inoculation success cannot be ascribed to the fungicide treatments. Furthermore, differences among treatment groups in final symptom development appear not to be attributable to inoculation success.

Occurrence and Progression of Crown Symptoms. By late August of the first year, localized foliar yellowing, abscision, or wilt occurred at most of the inoculation sites (Table 2, column B). In rate of occurrence of symptoms in the prophylactic groups, only Arbotect (40%) differed significantly ($P < .05$) from all other treatments 80-100%). The Arbotect treatment (70%) had the lowest rate of symptom occurrence among the therapeutic group (80-93%), but differences were not significant.

During the first year, symptoms progressed beyond the inoculated branch in 44% of the trees injected with water and 20% of the Phyton (HD) prophylactically-treated trees (Table 2, column C). Within the therapeutic group, symptom progression in 1985 occurred only in one (10%) Phyton (HD)-treated tree.

During the second year, 1986, symptoms con-

Table 2. Dutch Elm Disease Symptom Development Percent in Fungicide-Treated Elms Inoculated with *Ceratocystis ulmi*[a].

		DED symptom development (percent) *				
	Number of trees	*A C. ulmi cultured*	*B Foliar 1985*	*C Advanced 1985*	*D Foliar 1986*	*E Advanced 1986*
Prophylaxis						
Arbotect	10	60	40a	0	0a	0a
Fungi-Sol[c]	10	100	80ab	0	10ab	10a
Binab	10	100	80ab	0	10ab	10a
Phyton (ND)	11	82	91b	0	27bc	27ab
Phyton (HD)	10	90	90b	20	40bc	20ab
Water	9	78	100b	44	78c	67b
Therapy						
Arbotect	10**	80	70	0	0a	0a
Fungi-Sol	5	80	80	0	0a	0ab
Phyton (ND)	10	60	90	10	30ab	30ab
Phyton (HD)	5	100	100	0	40b	20ab
Binab	10	60	90	0	50b	40b
Water	9	78	100	44	78b	67b

* Data in columns followed by different letters are significantly different from other data within prophylaxis or therapy groups (Chi-square test, two-way comparison). Nine trees are listed with both prophylaxis and therapy groups. Symptom parameters are as follows:

A. Potato dextrose-agar cultures produced typical *Ceratocystis ulmi;* lack of positive culture does not negate the possible presence of viable fungus.
B. Symptoms (wilting, yellowing foilage, streaked sapwood) limited to the inoculated branches.
C. Symptoms in August 1985 in more branches than those inoculated.
D. All foliar symptoms in Sept. 1986.
E. Loss of much of crown and bole sapwood streaking.

** Five additional trees treated therapeutically with Fungi-sol in August 1986 all were severely diseased in September.

tinued to progress in all groups except Fungi-Sol therapy and both groups of Arbotect-treated trees (Table 2, column D). Among the prophylactic treatments, second year symptom progression was significantly less ($P < .05$) than in water controls (78%) in all treatments except the two Phyton dosages (27% and 40%, respectively). Among therapy groups, only the Arbotect treatment (0%) differed from the water injected prophylactic controls. The Fungi-Sol therapy group also had no diseased elms but the low number of trees given this treatment precluded statistical significance. Five additional elms inoculated and then treated with Fungi-Sol during August 1986 (not included in Table 2) all had severe foliar symptoms of DED in September 1986.

Morbidity and Recovery. A few of the trees in which foliar symptoms progressed during the second year appeared to have stabilized and, perhaps, to have recovered (Table 2, Column E). In the cases of the fungicide-injected elms ([two Phyton (HD) prophylactic, one Binab therapy, and one Phyton (HD) therapy], recovery might be ascribed to the treatment. However, one of the water controls also stabilized; dissection showed that, after about 15% of the crown died, the discolored area was compartmentalized and complete recovery seemed probable. Early spontaneous recovery of two additional water-injected trees can be inferred because the fungus had been cultured from these trees in 1985.

Injection Wound Closure. In one Phyton (ND), four Phyton (HD) and one Arbotect-treated tree, one or more injection wounds remained open and weeping (due to bacterial infection) in September 1986. Wounds on two of the Phyton (HD) treatments were associated with open dry lesions extending vertically for 10 cm or more above the injection holes. In all other fungicide-injected elms, wounds were closed and dry (Table 3).

Discolored areas associated with injections were greater at the ground level for all of the fungicide treatments than for water (Table 3; Figs. 2, 3). However, the discoloration was very localized after Fungi-Sol and Binab treatments. Arbotect injury extended to the 2m level in one of four dissected trees, but injuries were never associated with open lesions. Phyton (HD) treatments were relatively massive with 11.88% of the cross sectional stem area discolored at the root collar (0 level), 4.08% discolored at 1 m above ground, and discoloration extending to 3m. However, the most extensive discoloration (6 m) occurred in a tree injected with the lower Phyton

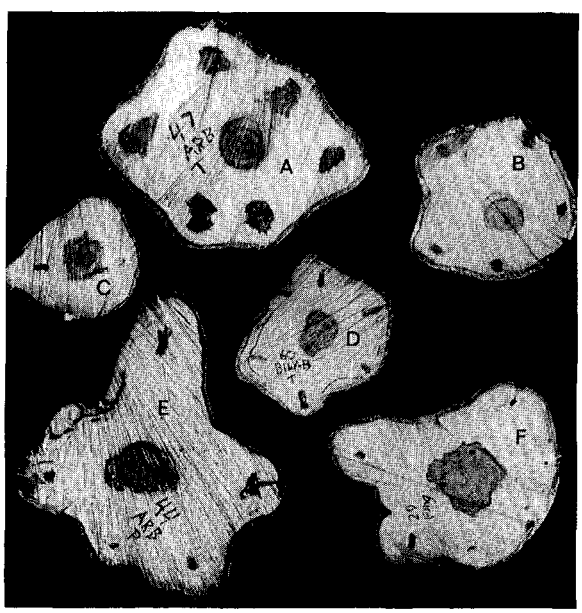

Fig. 2. Cross sections at root collars (level 0) of elms injected with various materials: A and E, Arbotect; B, Phyton (ND); C, water; D, Binab; F, Fungisol. In A and B discoloration of sapwood occurred laterally and tangentially from injection wounds; in other cases injury is limited to the vertical corridor. DED streaking is underlined in D.

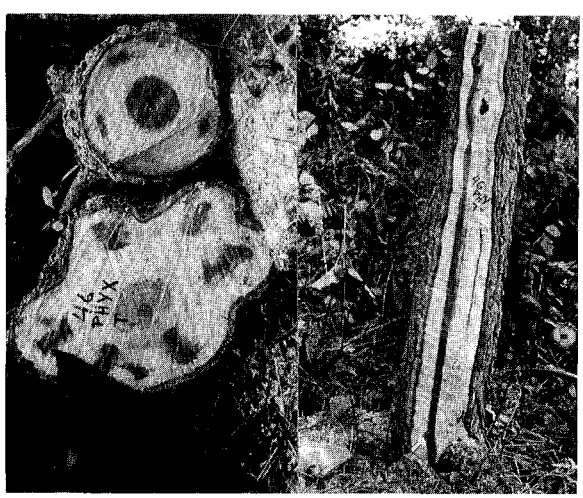

Fig. 3. Sections from a tree badly damaged by Phyton (HD). Left, cross sections at the root collar and 1 above; right, sapwood discoloration extending from injection point (arrow) through the first 1 m section of the tree.

(ND) dosage. As pointed out by Shigo and Campana (9), elms differ considerably in their reactions to the same fungicide treatment, however, it was clear that Phyton was the most damaging chemical, followed by Arbotect while treatments with Fungi-Sol and Binab were quite benign.

Foliar Toxicity. Arbotect injection was frequently followed by browning of edges of leaves on suckers and on some branches. Upper crowns of two Arbotect-treated trees partially exfoliated and showed limited branch tip mortality in 1985. However, all of the damaged crowns recovered and appeared healthy in 1986. None of the other treatments produced an immediate foliar reaction.

In some of the Phyton-injected trees, we noted as possible abnormal dying of low limbs. Foliage on these subordinant branches appeared normal in the fall of 1985 but a seemingly exceptionally high number failed to leaf out in 1986. However, this effect was not seen in open-crowned Phyton-treated trees so it might be a result of chance assignment of Phyton (HD) treatments to a disproportionate number of trees within dense canopies.

Discussion

Arbotect was clearly very effective against DED as a prophylactic or therapeutic treatment. This corroborates previous rigorous studies (3, 5, 11) demonstrating the usefulness of this material for combating DED. Possible therapeutic benefit of the other three materials is suggested but not statistically demonstrated by our results or any other published reports.

Fungi-Sol and Binab appeared to have significant prophylactic effect. However, DED symptoms in five additional elms innoculated early in June, treated therapeutically with Fungi-Sol in August 1986, appeared to be progressing in September. Fungi-Sol belongs to the fungicide class (benzimidazoles) that includes Arbotect and Lignasan so it could be expected to have some efficacy. At the low dosage of the active ingredient recommended by the Fungi-Sol manufacturer (0.016 gm/cm diam., 1/85th that of Arbotect) it is remarkable that any benefit is evident. Additional tests with greater concentration of Fungi-Sol and Binab are warranted. The method of delivery and effective dose of these materials may need adjustment.

Phyton performed poorly in both tests at both dosages. Injecting the treatment package (Phyton (HD)) into trees 17.8 cm (7") in diameter constituted a 13-fold greater concentration than would be present in a 25 inch diameter elm treated with the same material, yet fungicidal effect seemed marginal. Bole damage was unacceptably high for the low degree of protection gained. Lastly, the material was taken up very slowly. The 3-gallons used in Phyton (HD) required 1-3 full days to administer. Low dosage injections required about the same time as injection of 10-times the volume of Arbotect solution (i.e., .22 gal. Phyton vs. 2.0 gal. Arbotect solution in a 7-inch diameter tree).

The problem in treating trees on a diameter for-

Table 3. Injection wounds in dissected elms.

Treatment	*No. trees*	*Mean percent area discolored in bole cross sections*[a]							*Wound closure*[b]
		0	*1*	*2*	*3*	*4*	*5*	*6*	
Fungi-Sol	4	4.52	0	0	0	0	0	0	1.00
Binab	6	3.90	0	0	0	0	0	0	1.00
Phyton (ND)	4	4.82	2.37	1.50	1.78	1.38	1.52	0.3	1.75
Phyton (HD)	4	11.88	4.08	0.70	0.02	0	0	0	3.50
Arbotect	4	4.25	0.10	0.10	0	0	0	0	1.75
Water	7	1.34	0	0	0	0	0	0	1.00

a. Sections beginning at injection level in root collar (section 0) and taken at 1m intervals to 6m height.
b. Closure quantified for each tree as follows: 1 = closed, 2 = incompletely closed, dry, 3 = completely open, weeping, 4 = lesions 2 cm or less wide, 5 = lesions 3-4 cm wide, 6 = lesions 5 or more cm wide.

mula is well illustrated by our Arbotect injections. The dosage we used was only 1/4 that specified by the diameter formula of 12 oz./5 diameter inches. The phytotoxic effects we experienced suggest that small elms would be very badly damaged or killed by the label formula dosage. Dosages for systemic chemicals should be based on crown surface area projections such as that of O'Callaghan et al. (7) rather than strictly related to diameter.

Arbotect, Fungi-Sol and Phyton retard growth or kill *C. ulmi*. *Trichoderma viride* (in Binab) is anagonistic to *C. ulmi* in culture (8), but the mode of action within the elm tree is unknown. The very limited zone of discoloration around Binab injection holes indicates that the fungus did not colonize the tree. Any inhibition of *C. ulmi* would seem to result directly from *T. viride*-produced antibiotics that escape the compartmentalization zone or indirectly by the benign fungus stimulating the trees' generalized defensive reaction (i.e., production of phytoalexins) which, in turn, suppresses *C. ulmi* growth.

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