Influence of a Benzimidazole-Tolerant Isolate of *Ceratocystis ulmi* on the Control of Dutch Elm Disease with Methyl 2-Benzimidazole Carbamate Phosphate

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ABSTRACT

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American elm (*Ulmus americana*) seedlings 10.0–20.0 cm in diameter at breast height (dbh) were inoculated with either a benzimidazole-sensitive (WI) or a benzimidazole-tolerant (WIT) strain of *Ceratocystis ulmi*. When Dutch elm disease symptoms appeared, trees were injected with Lignasan BLP (methyl 2-benzimidazole carbamate phosphate) at either 0.8 or 4.0 g/2.5 cm dbh. Dutch elm disease symptoms were reduced below the control level only in trees inoculated with the WI strain and treated with Lignasan BLP at 4.0 g/2.5 cm dbh. Fungitoxicants were recovered from the symptom-bearing branches of a higher percentage of WI-inoculated elms than from WIT-inoculated elms. Decreased reisolation of the WI but not of the WIT strain occurred with increased fungicide concentration. The benzimidazole sensitivity of the WI and WIT strains remained stable, and the pathogenicity of the two strains did not differ significantly as measured by disease development from August 1976 through July 1978.

Additional key words: systemic fungicides.

The injection of solubilized benzimidazoles into elms has offered the only practical method of chemical control of Dutch elm disease (DED), caused by *Ceratocystis ulmi* (Buism.) C. Moreau (6–10, 14,16,17). The failure of benzimidazoles to control other plant diseases has followed the development of pathogen tolerance (1–4, 18,19) and has led to investigation of this problem as it pertains to DED. Various levels of tolerance of *C. ulmi* to benzimidazoles also have been reported (5,11–13,16), so it was desirable to determine, under field conditions and with recommended control procedures, whether the levels of tolerance in *C. ulmi* are sufficient to reduce control of DED in benzimidazole-treated elms.

Control of DED with benzimidazoles will be influenced by the frequency with which tolerant strains appear after exposure of the fungus to benzimidazoles in treated elms and by the stability and viability of such strains. Brasier and Gibbs (5) have shown that tolerant strains of *C. ulmi* were stable through 15 serial subcultures at 10-day intervals.

The level of pathogenicity of tolerant strains of *C. ulmi* will influence the significance of their role in the control of DED. In studies with chemically tolerant mutants or strains of plant-pathogenic fungi, some were as pathogenic as the wild types, while others were less so, and some had completely lost pathogenicity (2,18,19). Nishijima and Smalley (13) found more extensive vascular discoloration in 6-mo-old American elm seedlings inoculated with a tolerant strain than in those inoculated with a sensitive strain of *C. ulmi*.

This paper presents findings on: the influence of a benzimidazole-tolerant strain of *C. ulmi* on the effectiveness of chemotherapy of DED; the development and the stability of benzimidazole tolerance in vivo; and the comparative pathogenicity of a sensitive and a tolerant strain.

MATERIALS AND METHODS

A benzimidazole-tolerant variant (WIT) of a sensitive wild-type isolate (WI) of *C. ulmi* was selected after serial transfer of mycelium of WI onto potato dextrose agar (PDA) amended with 25 μg/ml (a.i.) of methyl 2-benzimidazole-carbamate hydrochloride (MBC-HCl). The WIT strain was isolated after the 10th transfer, and its level of tolerance was tested on PDA amended with either MBC-HCl or Lignasan BLP (methyl 2-benzimidazole carbamate phosphate) at 1, 5, 25, 100, and 500 μg/ml. All fungitoxicants in this study were sterilized by filtration through a Gelman Metricel filter GS-6 before addition to the agar. A drop of a standard spore suspension of either WI or WIT was pipetted onto a 12.7 cm Schleicher and Schuell filter paper pad in the center of amended or unamended PDA in a petri plate. There were four replicates of each treatment. The diameters of colonies growing on the agar were measured after 10 days.

American elm (*Ulmus americana* L.) seedlings, 10.0–20.0 cm diameter at breast height (dbh) were inoculated in a secondary branch with 0.5 ml of a suspension of 10^6 conidia per milliliter of either WI or WIT on 11 June 1976, by the method of Schreiber and Stipes (15). At the first appearance of crown symptoms, trees were injected (8) with Lignasan BLP at either 0.8 g (a.i.)/2.5 cm dbh (the label recommendation for therapy) or at 4.0 g (a.i.)/2.5 cm dbh. There were 36 trees inoculated with each isolate: 12 trees per Lignasan BLP treatment and 12 trees in the untreated control. All were arranged in a completely randomized design. Crown symptoms of DED were recorded on 5 August 1976; 1 July and 1 September 1977; and 19 July 1978.

Fungus reisolations were made on 1 October 1976 and 6 June and 4 October 1977 from branches with symptoms, and the benzimidazole tolerance of the reisolates was determined. Eight to 10 disks, 1 cm thick, from each branch sampled were flame sterilized with alcohol and placed onto acidified PDA amended with 0, 1, 3, 5, 25, 100, or 500 μg/ml (a.i.) Lignasan BLP. The percentage of trees from which the fungus was reisolated, and the levels of benzimidazole tolerance of the reisolates were determined for each treatment.

The same branches also were assayed for fungitoxicants. Wood disks were cut and sterilized as described above and placed on PDA seeded with sensitive *C. ulmi* conidia. After incubation of the plates at 24 C for 7–10 days, fungitoxicants were detected by the absence of fungal growth around or onto the disks.

RESULTS

The growth of both WI and WIT was inhibited more on PDA amended with Lignasan BLP than on PDA amended with MBC-HCl. Colony diameter of WI on MBC-HCl at 1 μg/ml reached 80% of the control value, but WI did not grow at all on Lignasan BLP at the same concentration or on either chemical at higher concentrations. Colony diameter of WIT on either chemical at 1–25 μg/ml equalled the control value. Colony diameter of WIT was 84 and 43% of the control value on agar amended with MBC-HCl at 100 and 500 μg/ml, respectively, but only 66 and 31% of the control value on agar amended with Lignasan BLP at 100 and 500 μg/ml, respectively.

Table 1 shows the average percentage of crown symptoms of DED in elms and the average percentage of elms from which sensitive (WI) and tolerant (WIT) strains of *C. ulmi* were reisolated after treatment with Lignasan BLP. In elms inoculated with WI, both rates of the fungicide significantly reduced crown symptoms 1 July 1977. On the two subsequent observation dates, crown symptoms were significantly reduced only by the higher concentration of Lignasan BLP. In elms inoculated with WIT, neither the 0.8 nor the 4.0 g/2.5 cm dbh treatment reduced disease symptoms in the crown at any time throughout the experiment. Crown symptoms in untreated trees inoculated with WI did not differ significantly from those in untreated trees inoculated with WIT.

Fungus reisolation data from the three sampling dates have been combined. The percentage of trees from which WI was reisolated decreased with increasing chemical concentration and was significantly lower in trees treated with either chemical concentration than in control trees. The percentage of treated trees from which WIT was reisolated did not differ significantly from the control.

Only one of the reisolates from either treated or untreated trees showed any change in fungicide tolerance during the course of the experiment. The exception was the reisolation of a tolerant strain of WI from an elm treated with Lignasan BLP at 0.8 g/2.5 cm. The reisolate grew on PDA amended with 100 μg/ml Lignasan BLP. Reisolates obtained subsequently from the same tree were sensitive and did not grow at 1 μg/ml Lignasan BLP.

Assay data for fungitoxicants in branches with symptoms were combined for both chemical treatments on the three sampling dates. Fungitoxicants were detected in a significantly higher percentage of treated WI-inoculated trees (56%) than treated WIT-inoculated trees (17%). No fungitoxicants were detected in untreated trees.

DISCUSSION

Our data show that a concentration of Lignasan BLP five times that recommended for therapeutic treatment did not control DED in trees infected with a tolerant strain (WIT) of *C. ulmi*. Equally important, we found that even in trees inoculated with a sensitive (WI) strain, the recommended rate of Lignasan BLP only briefly retarded disease development.

The lack of disease control in trees inoculated with WIT probably was influenced by the high level of tolerance of the strain (in excess of 500 μg/ml). Although surveys of natural populations of *C. ulmi* by some workers indicate tolerance levels below 10 μg/ml (5,11), others (12,13,16) have found strains with far higher tolerance levels. The tolerance level of WIT is within the range of tolerance found in nature.

TABLE 1. Percentage of crown symptoms of Dutch elm disease in American elm and percentage of elms from which benzimidazole-sensitive (WI) or tolerant (WIT) strains of *Ceratocystis ulmi* were reisolated after treatment with Lignasan BLP

Strain inoculated[b]	Lignasan BLP treatment rate (g/2.5 cm dbh)[c]	(%) Crown symptoms[a] 5 August 1976	1 July 1977	1 September 1977	19 July 1978	*C. ulmi* reisolation (% of elms)[d]
WIT	4.0	47 z	80 zy	85 z	95 zy	70 z
	0.8	55 z	75 zy	90 z	100 z	48 z
	Control	46 z	74 zy	74 z	92 zy	75 z
WI	4.0	35 z	38 x	45 y	77 y	17 y
	0.8	38 z	64 y	78 z	86 zy	38 y
	Control	40 z	91 z	95 z	100 z	77 z

[a]Within a column, differences between numbers followed by the same letter are not statistically significant ($P = 0.05$).
[b]Inoculations made 11 June 1976.
[c]Trunk injections with Lignasan BLP were made following appearance of the first crown symptoms.
[d]Percentages of elms represent combined data from isolations on 1 October 1976, and 6 June and 4 October 1977.

The level of pathogenicity of the tolerant strains will affect their impact on disease control. Although the tolerant strain appeared to be less pathogenic than the sensitive strain, the difference was not statistically significant. Other evidence (2,13,18,19) indicates that tolerant strains of other pathogens may vary in pathogenicity either above or below that of the sensitive ones.

We found, as did others (5,13), that tolerant strains do not readily revert to the sensitive condition. On the other hand, we found that sensitive strains in trees treated with Lignasan BLP did not readily produce tolerant variants even after two and one-half growing seasons. The single reisolation of a tolerant culture from a tree inoculated with sensitive strain may be only a random event not influenced by the presence of Lignasan BLP in the tree.

The lower level of funitoxicity in branches of trees inoculated with WIT may be a direct result of the increased disease caused by this strain with vascular blockage leading to reduced movement of the chemical into the crown.

Finally, with other plant pathogens, control failure due to the presence of tolerant strains has occurred under field or storage conditions. In such cases, large segments of the fungal population are exposed to chemical treatment because of the size of the areas treated, the proliferation of the pathogens, or both. Such extensive exposure does not occur when elms are treated for DED, for two reasons. First, a relatively small percentage of infected elms are treated because of high treatment costs. Second, fungus-chemical interaction may be limited by sparse fungus reproduction in the elm and by restricted chemical and fungus distribution caused by tyloses and gummosis in the vessels.

It is still too early to predict the full impact of tolerant strains of *C. ulmi* on the use of benzimidazoles to control DED. However, we have shown that Lignasan BLP injected at up to five times the recommended therapeutic dosage does not control DED in American elms infected with a tolerant strain of *C. ulmi*. Also, stability of the tolerant characteristic and pathogenicity of the tolerant strain suggests that DED may be difficult to control chemically. While tolerant strains appear to be relatively rare, the frequency with which they develop in the future will influence prospects for chemical control.

LITERATURE CITED

1. BEN-YEPHET, Y., Y. HENIS, and A. DINOOR. 1974. Genetic studies on tolerance of carboxin and benomyl at the sexual phase of *Ustilago hordei*. Phytopathology 64:51-56.
2. BERTRAND, P. F., and J. L. SAULIE-CARTER. 1978. The occurrence of benomyl-tolerant strains of *Penicillium expansum* and *Botrytis cinerea* in the mid-Columbia Region of Oregon and Washington. Plant Dis. Rep. 62:302-305.
3. BOLLEN, G. J., and G. SCHOLTEN. 1971. Acquired resistance to benomyl and some other systemic fungicides in a strain of *Botrytis cinerea* in cyclamen. Neth. J. Plant Pathol. 77:83-90.
4. BOLLEN, G. J., and A. VAN ZAAYEN. 1975. Resistance to benzimidazole fungicides in pathogenic strains of *Verticillium fungicola*. Neth. J. Plant Pathol. 81:157-167.
5. BRASIER, C. M., and J. N. GIBBS. 1975. MBC tolerance in aggressive and non-aggressive isolates of *Ceratocystis ulmi*. Ann. Appl. Biol. 80:231-235.
6. CAMPANA, R. J., G. GREGORY, and T. JONES. 1973. Arrest of symptom development in Dutch elm disease with pressure injection of solubilized benomyl. 11th Int. Congr. Plant Pathol., Minneapolis, Minn.
7. GIBBS, J. N., and J. DICKINSON. 1975. Fungicide injection for the control of Dutch elm disease. Forestry 48:165-176.
8. GREGORY, G. F., and T. W. JONES. 1975. An improved apparatus for pressure-injecting fluids into trees. U.S. For. Serv., Res. Note NE-214.
9. JANUTOLO, D. B., and R. J. STIPES. 1976. Toxic effects of methyl 2-benzimidazole carbamate (MBC), MBC-HCl, MBC-H_2PO_4 and nystatin to *Ceratocystis ulmi*. Can. J. Plant Sci. 56:967-970.
10. KONDO, E. S. 1977. A six-year summary of four years of field experiments with MBC-P solutions to control Dutch elm disease. Can. For. Serv. Bi-mon. Res. Notes 33:22-24.
11. KONDO, E. S. 1978. Scope and limitations of carbendazim. H_3PO_4 injections in Dutch elm disease control. J. Arboric. 4(4):80-86.
12. NISHIJIMA, W. 1976. Fungicides for Dutch elm disease. Ph.D. Thesis, Univ. of Wisconsin, Madision. 125 pp.
13. NISHIJIMA, W. T., and E. B. SMALLEY. 1979. *Ceratocystis ulmi* tolerance to methyl 2-benzimidazole carbamate and other related fungicides. Phytopathology 69:69-73.
14. SCHREIBER, L. R., T. W. JONES, and G. F. GREGORY. 1978. Control of Dutch elm disease: comparison of benomyl and methyl 2-benzimidazole carbamate hydrochloride and two injection techniques. Plant Dis. Rep. 62:761-765.
15. SCHREIBER, L. R., and R. J. STIPES. 1966. A method for the inoculation of elms with a spore suspension of *Ceratocystis ulmi*. Plant Dis. Rep. 50:808-810.
16. SCHREIBER, L. R. and A. M. TOWNSEND. 1976. Naturally occurring tolerance in isolates of *Ceratocystis ulmi* to methyl 2-benzimidazole carbamate hydrochloride. Phytopathology 66:225-227.
17. VAN ALFEN, N. K., and G. S. WALTON. 1974. Pressure injection of benomyl and methyl 2-benzimidazole carbamate hydrochloride for control of Dutch elm disease. Phytopathology 64:1231-1234.
18. VAN TUYL, J. M. 1977. Genetics of fungal resistance to systemic fungicides. Meded. Landbouwhogesch., Wageningen, The Netherlands. 136 pp.
19. WUEST, P. J., H. COLE, Jr., and P. L. SANDERS. 1974. Tolerance of *Verticillium malthousei* to benomyl. Phytopathology 64:331-334.