TOLERANCE OF THE DUTCH ELM DISEASE FUNGUS CERATOCYSTIS ULMI TO SOLUBILIZED BENOMYL[1]

by Richard J. Campana and Barbara L. Schafer

Introduction

The only chemical in the long history of testing to be consistently effective against Dutch elm disease is benomyl or one of its derivatives. Benomyl (Benlate 50 W, the wettable powder) is currently one of the most effective chemicals for fungus control of plant disease. Benlate in water suspension has been effective to some degree against Dutch elm disease when applied as a soil amendment (Biehn and Dimond 1970), or as a foliar spray (Hart 1972; Smalley 1973). However, Van Alfen (1974) did not find it effective in direct stem injection. It's derivatives, methyl 2-benzimidazole in hydrochloride (MBC•HCl), methyl 2 benzimidazole phosphate (Lignasan BLP), or 2-(4-thiazolyl)-benzimidazole (Mertect) solubilized in water have been effective in preventing infection or arresting disease progress when injected directly into stems (Campana, Gregory and Jones 1973; Gibbs and Clifford 1974; King and Campana 1973; Kondo 1972; Smalley 1971; Stipes 1973). However, there are many reports on field tolerance to Benlate by several plant pathogens (Bollen and Scholten 1971; Georgopoulos and Dovas 1973; Miller and Fletcher 1974).

Thus, it was expected that tolerance to MBC might develop in *C. ulmi*, and several studies were made to test for such tolerance (Brasier and Gibbs 1975; Hindal 1975; Schafer 1976; Schafer and Campana 1976; Schreiber and Hindal 1976; Schreiber and Townsend 1976). The recent paper by Schreiber and Townsend (1976) is concerned with natural tolerance prior to any influence by benomyl or any of its derivatives. The only publication on development of tolerance *per se* of *C. ulmi* after exposure to any of the benomyl derivatives is that of Brasier and Gibbs (1975). Before this paper was published, another study (the fungicidal-fingistatic effect of benomyl derivatives on *C. ulmi* conidia [Janutolo & Stipes] 1975]) was reported to be in progress. This paper is limited to an evaluation of two independent studies on tolerance (Brasier and Gibbs 1975; Schafer 1976).

The concept of tolerance

Tolerance is best seen as a dynamic phenomenon. It is not only the ability of a pathogen to survive a toxic chemical; in addition, it represents the capacity to survive increasingly greater dosages. This requires the ability to change through mutation, or sexual variation. Since every living organism has the capacity to change, each individual has evolved what may be termed a natural rate of mutation in the environment where it evolved. Tolerance implies change through mutation, or selection within a genetically diverse population under a particular stress where the stress exerts a selective pressure. Individuals sensitive to that stress expire, leaving the "tolerant" ones to survive and reproduce tolerant individuals. However, tolerance may represent current or naturally-occurring resistance as noted by Schreiber and Townsend (1976), or it may even be seen as survival of an individual spore under chemical stress, with development after the stress terminates.

Effective use of benomyl

For at least 30 years, plant pathologists have sought in vain to find a suitable systemic fungicide that would be effective in preventing or arresting infections of Dutch elm disease. For almost 30 years, one chemical after another looked promising in laboratory studies but failed in field tests. Only six years ago did we learn that benomyl in the form of Benlate 50W could be effective in limiting infections in elms (Biehn and

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Dimond, 1970). We have since learned that the Benlate form of benomyl has serious limitations for disease control under field conditions. Only four years ago, many plant pathologists "solubilized benomyl" *sensu* Stipes (1973-UIFRO paper) could be effective for prevention, as well as for therapy of trees already diseased. And now, with several years of data on which to draw, we have reached a new plateau, where we can point to hundreds of trees now apparently free of disease that were once infected; and we can speculate on how many trees that may have been saved from infection by chemical injection. But from this same vantage point, we can see many failures; we can see recently-injected trees, ostensibly healthy but still infected, that will collapse next year, or the year after, from infections currently suppressed, while the fungus waits for the chemical to ease off. Thus it is evident, that in spite of early success with solubilized benomyl, we still have a long way to go. We must examine the limitations of the new chemicals as well as of chemical injection, and see how and where we can improve the record. My comment here is essentially for trees already infected, but may have relevance also to healthy trees injected for prevention.

Limitations of chemical injection

In spite of early success with chemical treatment in preventing or arresting Dutch elm disease, experience and insight reveal various limitations in effectiveness that cannot be ignored:

1. Solubilized benomyl does not often kill the fungus once established in living tissues (Campana, Gregory and Jones 1973; King and Campana 1974). This chemical with fungicidal properties at specific levels, acts more as a *fungistat* at levels where it contacts the fungus in injected trees (Janutolo & Stipes, 1975).

2. Solubilized chemicals do not move into heavily-infected tissues, even under pressure, for the same reason that wilting occurs; i.e., the water-conducting vessels are blocked. Thus, the chemical moves only into tissues not yet invaded or lightly invaded by the pathogen. Thus, even in therapy, the chemical acts functionally as a preventative.

3. From trunk injections, solubilized benomyl does not become uniformly distributed in small branches where infection from insect wounds begin. Even with pressure, detectable chemical in small branches of small trees is at best about 80 percent; in large trees, at best it is about 40-60 percent. Thus each large tree injected has extensive branch areas in its crown where the chemical is likely to be absent.

4. Effective dosage per tree has never yet been documented. In addition, effective dosages at points of introduction may be diluted to ineffectiveness in small stems unless the dosage at introduction is adequate to compensate for dilution. Little is currently known concerning rates of absorption on plant cells or dilution by systemic movement through thousands of branching vessels. Thus, the chemical must be as highly concentrated as is possible at the point of introduction (Campana 1969).

5. Strains of the Dutch elm disease fungus may develop tolerance to solubilized chemicals even within an individual tree. In a large sense, unless all of the spores of the pathogen are killed on contact with the chemical, the fungus is to some degree tolerant. This is the area with which this paper is concerned.

6. Also, there are already in existence naturally, tolerant strains of the pathogen; i.e., tolerant strains never before exposed to any of the benomyl compounds.

Methods used in testing for tolerance

MBC•HCl was processed as outlined by McWain and Gregory (1971). In effect, through a series of solubilizations, heating, filtration, pH adjustment, resolubilization and dilution, a clear tan solution of MBC in HCl with a low pH at about 72 g/l was obtained for further dilution. At a concentration of 6 g/l this solution had been shown to be effective in arresting or preventing symptoms of Dutch elm disease (Campana, Gregory and Jones 1973; Gregory and Jones 1974; King and Campana 1974) without killing the fungus. The asexual-state tests involved growing mycelial discs of the fungus on agar amended with various levels of the chemical. The most vigorous isolates were transferred weekly over 15-26 weeks to plates with successively higher levels

of the chemical. The isolates were then returned to agar without any chemical. The sexual-state tests involved harvesting of ascospores from fruiting bodies (perithecia) formed by crossing compatible mating strains (Rosinski 1960). They were exposed to various levels of chemical as with mycelial discs. Testing of inheritance of tolerance involved crossing compatible mating strains of sensitive with tolerant isolates, and harvesting ascospores to determine tolerance of the progeny on agar with various levels of the chemical.

Wild isolates of the pathogen were typed by use of a numerical rating system by comparing them with standard cultures field tested for pathogenicity by Dr. S. McNabb, Iowa S.U. (Schafer and Campana 1976). The rating system was used to identify aggressive or weak isolates by observation in culture of: 1) linear radial growth rate; 2) amount of aerial growth (*mycelium*); 3) variation in daily growth (diurnal variation); and 4) variation in radial growth (*radial striation*). Isolates with high numerical values were considered aggressive; those with low values, weak; those with intermediate values were rated neither aggressive nor weak. Confirmation of these ratings has not yet been tested in field studies.

Major findings from English and American tolerance studies

A comparative evaluation of factors relative to tolerance is presented in Table 1. Development of tolerance in both studies was not correlated (-) to pathogenicity (capacity to cause disease) or mating type of *C. ulmi.* Tolerance developed equally in both aggressive and non-aggressive isolates, as well as in both mating types of the pathogen in both studies. However, Schafer (1976, American study) found that some aggressive isolates were only temporarily reduced to non-aggressiveness by exposure to the chemical. Non-aggressive isolates were less sensitive to change under similar exposure.

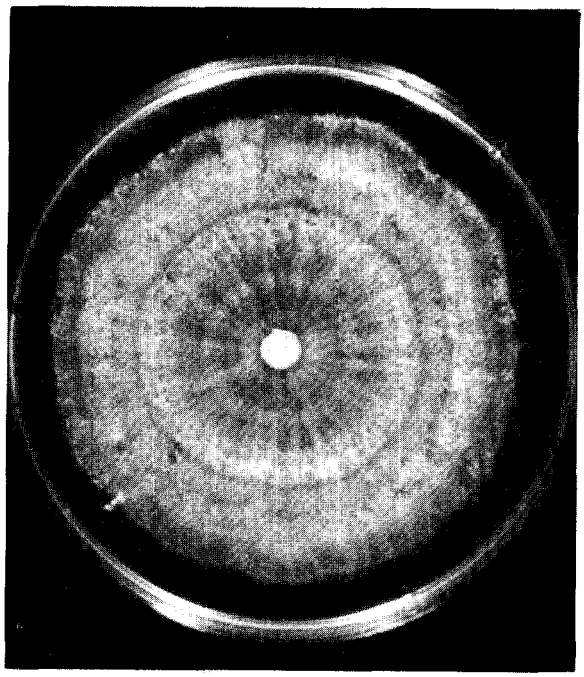
Both Schafer (1976) and Brasier and Gibbs (1975, English study) found a similar frequency in mutation to tolerance among asexual spores. Both studies on *C. ulmi* showed the tolerance of wild strains of both aggressive and non-aggressive isolates to be significantly less than that following successive exposure to MBC•HCl (Fig. 1). Whether the frequency of mutation found in the studies in asexual spores represents frequency of naturally-occurring mutations as a normal phenomenon, or results from mutagenic effect of MBC•HCl, is not known (Brasier and Gibbs 1975; Schafer 1976).

Since Brasier and Gibbs (1975) did not evaluate tolerance in sexual spores, Schafer's data is the only information here. In her study cultures derived from sexual spores (ascospores) exposed to MBC-HCl showed a 12-fold increase in tolerance to MBC•HCl over those of asexual origin similarly exposed. Thus, through sexual reproduction, there was an increased capacity for tolerance to the chemical. This was not unexpected, since tolerance was expected to develop under selection pressure. These are the only data on tolerance of ascospores to *C. ulmi* of which we are aware (Schafer 1976). Tolerant isolates inhibited by MBC•HCl, whether of asexual or sexual origin, resumed normal growth when released from influence of the chemical.

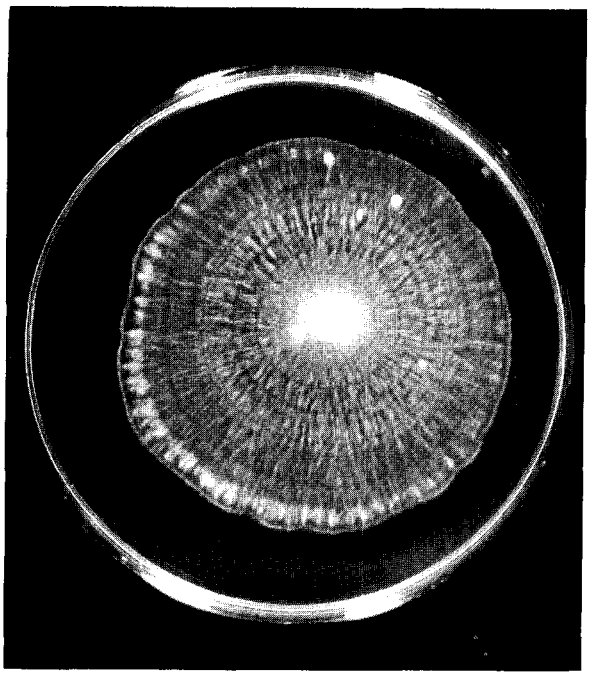
In effect, the chemical appeared to convert aggressive strains to non-aggressive ones, at least on a temporary basis. However, in all strains exhibiting tolerance, some stability was retained after exposure to the chemical was removed. Cultures derived from sexual crosses were significantly more stable while those derived from asexual sources were less so. The evidence is clear here; the tolerance acquired by strains of non-sexual origin often is temporary, with no permanent alteration of genes; those strains from spores of sexual origin apparently represent genetic change toward tolerance. In summary, the effect of the chemical under the conditions tested here was fungistatic rather than fungicidal. This is in consistent agreement with field observations by many investigators.

Significance of findings in tolerance studies

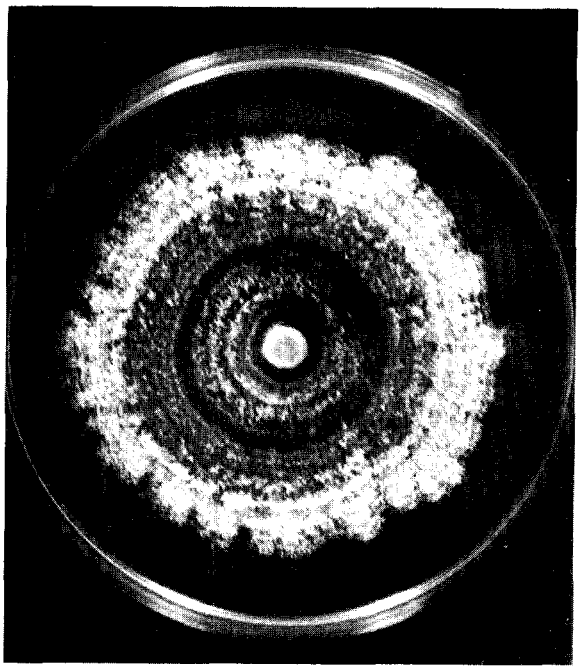
The ease by which tolerance to MBC•HCl in *C. ulmi* can develop under laboratory conditions, suggests that field tolerance may develop with similar ease. But laboratory demonstration of



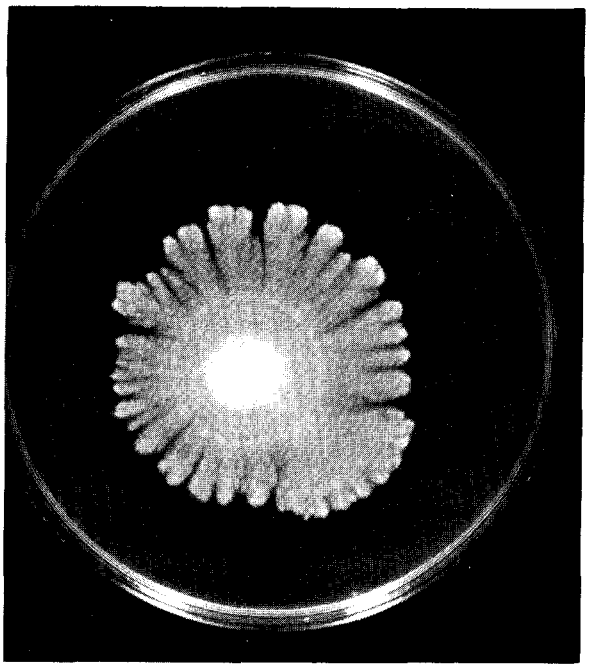
A. wild strain—aggressive



B. wild strain—nonaggressive



C. aggressive (A) following exposure to MBC•HCl



D. nonaggressive (B) following exposure to MBC•HCl

Fig. 1. Aggressive and nonaggressive strains of *C. ulmi* (grown on malt extract agar before and after exposure to MBC•HCl).

Table 1. Comparative evaluation of two studies (English and American) of tolerance of *Ceratocystis ulmi* to methyl-2-benzimidazole carbamate by relative factors.

Factors relative to tolerance	Brazier & Gibbs (English, 1975)	Schafer (American 1976)
1. Pathogenicity	-	-
2. Mating	-	-
3. Asexual stage Frequency[a] (Conidia)	+ $1\text{-}1.3 \times 10^8$	+ $1\text{-}1.3 \times 10^8$
4. Sexual stage Frequency[b] (ascospores)	Not tested	$1\text{-}1.9 \times 10^8$
5. Mendelian segregation	+	+
6. Stability of tolerant mutations	+	+

[a] 1 in 130,000,000
[b] 1 in 190,000,000

capacity for tolerance does not prove that similar tolerance will occur in the field. However, that tolerance may develop by chemical exposure in the laboratory to either asexual or sexual spores is not to be taken lightly. We have already noted a degree of tolerance in the survival of the fungus in trees where symptoms have been arrested (Campana, *et al.* 1973), or coexistence of the fungus with MBC•HCl in the same tissues (King and Campana 1974; Sior and Campana 1975). And there is now evidence of natural tolerance not induced by chemical (Schreiber and Townsend 1976).

In branches where the chemical is not present at levels sufficient to inhibit growth completely, the development and downward movement of spores in vessels is certain. At some level between infection high in the crown and chemical injection at the base of the tree, the fungus and chemical may contact one another at levels conducive to development of fungus mutations.

We have seen that some mutations may be stable where isolates do not revert to their original sensitivity. Other sensitive isolates may develop when the chemical is absent or diluted below inhibitory levels. We are now at the point where we must attempt to correlate laboratory and field studies on tolerance, and study the possibility of increased tolerance in isolates from chemically-injected trees.

Therefore, given incomplete protection, the magnitude of spore production in vessels, and the movement patterns of spores and injected chemicals, the development of tolerance within individual trees seems possible and probable. This could have a significant influence on the effectiveness of chemical injection.

Brasier and Gibbs (1975) suggested that chemically-induced tolerant strains may not compete successfully with sensitive ones. Schreiber and Townsend (1976) indicate that naturally existing tolerant strains may be just as competitive. From this, one may infer a proportional rise in frequencies of such strains as beneficiaries of the selective pressure of chemical injection. This may be so, but needs exploration. Brasier and Gibbs (1975) suggested that probability of chemically-induced development of tolerance was not likely by sexual means, because of the complexities required for insect mixing of compatible mating strains in their breeding activities. This also should be explored. The situation is obviously too complex to be resolved easily or quickly. At this point, from current evidence on the development of tolerance, we can only stress that there may be increased failure to prevent or arrest the disease in particular trees; and thus we must seek more understanding on tolerance of *C. ulmi* to chemicals used for disease prevention or therapy.

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ABSTRACT

Odell, T.M. and I.H. von Lindern. 1976. **A technique for marking first-stage larvae of the gypsy moth for dispersal studies.** USDA Forest Service Research Note NE-223, 4 p.

Wind dispersal of newly-hatched larvae of the gypsy moth is a major factor in the geographical spread of this pest. Although this means of distribution was noted in the early 1900s, only recently have studies been initiated to identify the morphological and meteorological characteristics associated with air-borne distribution of the tiny first-stage larvae. One of the problems associated with identifying the characteristics of the aerobiological pathways of dispersal was the lack of an adequate technique for correlating dispersed larvae with any particular point source. Development of such a technique requires an appropriate tagging or tracing element that will not adversely affect the relatively fragile insect and can be detected easily in the field. Zinc cadmium sulfide fluorescent particles can be used to mark first-stage larvae of the gypsy moth without effecting changes in their development and behavior. Marked larvae dispersed readily; so the technique could be used to correlate dispersed larvae with any particular source point.