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SHORT PAPER

Tolerance of aggressive and non-aggressive isolates of *Ceratocystis ulmi* to organotin fungicides

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The effect of triorganotin compounds, R_3SnX, on the growth of three wild strains of *Ceratocystis ulmi (C. ulmi)* fungus, two aggressive and one non-aggressive strains, was evaluated in shake culture. In all cases, the triphenyltins were the more effective organotins for the inhibition of *C. ulmi in vitro*. The anionic group, X, did not have a significant role in the inhibition, suggesting that the species involved in the inhibition is the triphenyltin moiety (Ph_3Sn^+) or the hydrated triphenyltin moiety ($Ph_3Sn(H_2O)_2^+$). It is further suggested that the triphenyltin species Ph_3SnOH and Ph_3SnOAc are the preferred compounds for the control of Dutch elm disease. The tolerance of aggressive isolates to fungitoxins appears to depend more on the nature of the fungicide than on the type of fungus.

Keywords: *Ceratocystis ulmi*, fungi, aggressive and non-aggressive strains, wild strains, Dutch elm disease, organotins, triphenyltins, tricyclohexyltin, fungicide

INTRODUCTION

Dutch elm disease (DED), one of the most serious plant diseases of the 20th century, is caused by the fungus *Ceratocystis ulmi*. It was first observed in the Cleveland, Ohio, USA, area in 1930,[1] and within the next 60 years has spread virtually throughout the United States.[1] This disease has killed millions of elm trees in Europe and North America and has caused incalculable damage both economically and environmentally.[1] The current fungicides used in the control of DED have met with limited success.[2–4] Thus the development of a more effective fungicide to combat DED would be most valuable. There have been numerous studies on the biological properties of organotin compounds.[2–5] Generally, it has been concluded that the biocidal effects of these compounds are a function of the organic moiety as well as the number of organic groups attached to the tin atom.[2–5] In the interest of developing a more effective fungicide against *Ceratocystis ulmi*, the causative agent of Dutch elm disease, our laboratory has screened a host of organotin compounds against this fungus.[6]

The results indicated that the most effective triorganotin compounds (R_3SnX) were those that contained the tributyl, tricyclohexyl and triphenyltin moieties.[6] However, the user of organotin compounds as agricultural fungicides must consider not only their fungicidal activities but also their phytotoxicities. Thus, the tributyltin compounds can be excluded as an agricultural fungicide due to their high phytotoxicities.[7,8] It was further observed that the anionic group attached to the tin atom did not have any significant effects in our initial inhibition studies.[6] A later study using triphenyltin halide adducts, $Ph_3SnX \cdot L$, where X = Cl or Br, and L = Me_2SO, $PhCONH_2$ or Ph_3PO, as the toxicant showed again that the group X and/or the ligand did not play a major role in the inhibitory activities of these adducts. This suggests that the species involved in the inhibition is the Ph_3Sn^+ ion or the hydrated cation, which is in agreement with our earlier studies.[6,9]

Gibbs and Brasier[10] determined that there are two culturally distinguishable strains of *Ceratocystis ulmi* which they designated as aggressive and non-aggressive. Janutolo and Stipes,[11] testing the response of aggressive and non-aggressive isolates of *Ceratocystis ulmi* to

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benzimidazole carbamate fungitoxicants, concluded that the aggressive isolates were more tolerant to the fungitoxicants than the non-aggressive strains.

Therefore, before organotin compounds can be used for the control of DED, their effectiveness against both aggressive and non-aggressive strains of the fungus must be investigated. The present study reports the tolerance of three wild strains of *Ceratocystis ulmi* to triphenyl- and tricyclohexyltin compounds.

EXPERIMENTAL

Chemicals

With the exception of triphenyltin bromide and iodide, the organotin compounds were purchased commercially. Triphenyltin acetate and triphenyltin hydroxide were purchased from Alfa Products (Danvers, MA), and triphenyltin chloride was obtained from Aldrich Chemical Company Inc. (Milwaukee, WI). The tricyclohexyltins were purchased from Organometallics, Inc. (E. Hampstead, NH). All the compounds were used as received without further purification. The triphenyltin bromide and iodide were synthesized according to the procedures of Chambers and Scherer.[12] They were recrystallized from light petroleum distillate and their melting points were in agreement with literature values.

Elemental analysis

The elemental analysis were performed by Schwarzkopf Microanalytical Laboratory, Woodside, New York.

Preparation of stock organotin solution

The compounds to be screened against *Ceratocystis ulmi* were each dissolved in ethyl alcohol to give a final concentration of 100 mg dm^{-3}. Appropriate volumes of the toxicant were then incorporated into the test solutions to give the desired concentrations.

The fungus

The wild strains of *Ceratocystis ulmi* were obtained from Dr L Frederick of the Department of Botany, Howard University, Washington, DC, USA, and the preparation of the fungus for the toxicity studies has been previously described.[6]

Fungicidal activity

The procedure for evaluating the fungicidal activity for the various organotins has been previously described.[6]

RESULTS AND DISCUSSION

Two series of organotins, phenyltin and cyclohexyltin derivatives, were screened *in vitro* against three wild strains of *Ceratocystis ulmi*, two aggressive strains (DC-03 and DC-05B) from Washington, DC, and one non-aggressive strain (NH-6) from New Hampshire. The results of the bioassay along with the previous results obtained by using the American Type Culture Collection (ATCC) strain 32434 in a shake culture medium are summarized in Table 1.

The results of the minimum inhibitory concentration (MIC), the concentration at which 50% of the species are inhibited, *in vitro* screening studies against all three wild strains of *Ceratocystis ulmi* indicate that the phenyltins as a class were more effective than the tricyclohexytins. This is in agreement with the earlier studies using the ATCC strain 32437.[6,9]

The inhibitory effect of the triphenyltin compounds was highest for the aggressive strain, DC-05B, followed by the non-aggressive strain NH-6, with the aggressive strain, DC-03, showing the least inhibition. Because of the limited number of strains tested, no definitive pattern was observed as to whether the aggressive isolates are more tolerant to the organotin fungitoxins than the non-aggressive isolates. This contrasts with the work of Janutolo and Stipes,[11] who tested three aggressive and three non-aggressive strains using a benzimidazole fungicide. They found that all of the aggressive strains of *Ceratocystis ulmi* were more tolerant to benzimidazole carbamate phosphate than their non-aggressive isolates.[11] However, our results would suggest that the tolerance of aggressive strains to fungitoxins may be more attributable to the specificity of the fungitoxin itself and may not be dependent upon the type of fungus used as reported by Janutolo and Stipes.[11]

The fungicidal activity of the tricyclohexyltins is also considerable in its own right, as shown from this study. A comparison of the tricyclohexyltin compounds tested showed that the three compounds screened in this study were equally effective against strain DC-05B and least effective

Table 1 Minimal inhibitory concentration (MIC) of some organotin compounds against three wild strains of *Ceratocystis ulmi* in potato dextrose broth at 22°C

	MIC (mg dm^{-3})			
	Strain			
Compound	ATCC 32437[a]	NH-6 (non-aggressive)	DC-03 (aggressive)	DC-05B (aggressive)
Phenyltins				
$(C_6H_5)_3SnBr$	2	3	4	2
$(C_6H_5)_3SnI$	2	3	5	2–3
$(C_6H_5)_3SnCl$	2	3	4	2
$(C_6H_5)_3SnOOCCH_3$	2	3	4	2
$(C_6H_5)_3SnOH$	2	3	5	2
Cyclohexyltins				
$(C_6H_{11})_3SnBr$	5	15	20	5
$(C_6H_{11})_3SnCl$	10	15	20	5
$(C_6H_{11})_3SnOH$	10	10–15	20	5

[a]From Ref. 6.

against the DC-03 strain. While the phenyltins, as a class, are more effective in the inhibition of *Ceratocystis ulmi* than their cyclohexyl analogs, the latter compounds should not be excluded as possible controlling agents for DED. The primary advantages of the tricyclohexyltins lie in their low phyto- and mammalian toxicities,[7] assuming that they can be taken up and translocated. As with the phenyltins, no pattern was observed as to which isolate (aggressive or non-aggressive) is more tolerant to the fungitoxicants.

The fact that the triphenyltin compounds have similar inhibitory effects against the three strains of *Ceratocystis ulmi* tested supports the earlier conclusion that the anionic group X does not play a major role in the biological activity of these compounds.[6,9] Thus, the observed inhibition of the three wild isolates of *Ceratocystis ulmi* by the triphenyltin compounds is probably due to the formation of either the $(C_6H_5)_3Sn^+$ species or its hydrated analog, $(C_6H_5)_3Sn(H_2O)_2^+$.

An effective organotin fungicide for the controlling of DED should be a compound that has high fungicidal activity and low phytotoxicity. Ascher and Nissim[13] and Pieters[14] observed that the phytotoxicity of Ph_3SnX compounds was influenced by the nature of the X group. Phytotoxicity was found to be highest when X was either a chloro or a sulfate group and least when X was either an acetate or a hydroxide group. In view of these observations and the results from our studies, it appears that $(C_6H_5)_3SnOH$ and/or $(C_6H_5)_3SnOOCCH_3$ would be the preferred organotin fungicide for the controlling of DED.

It is still too early to consider the use of organotins to control DED. Additional studies involving translocation, biodegradability and phytotoxicity must be compiled and evaluated before these compounds can be recommended for potential DED control. However, we have shown that if organotins are the compounds of choice, triphenyltins or one of their derivatives should be used, regardless of whether the strain of *Ceratocystis ulmi* is aggressive or non-aggressive.

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