Cross-Resistance in *Cercospora beticola* to Triphenyltin and Oligomycin

M. CHRYSAYI-TOKOUSBALIDES and C. N. GIANNOPOLITIS, Benaki Phytopathological Institute, Kiphissia, Athens, Greece

ABSTRACT

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Triphenyltin-resistant strains of *Cercospora beticola* isolated from naturally infected sugar beets were also resistant to oligomycin in vitro. This correlation between resistance to triphenyltin and to oligomycin is independent of benzimidazole resistance. The finding of cross-resistance between triphenyltins and oligomycin suggests a similarity in the mode of action of these compounds and supports the hypothesis of a genetic basis for the development of resistance to triphenyltins.

Additional key word: carbendazim

The mechanism of action of trialkyltin and triphenyltin compounds has been proposed to involve the inhibition of ATP formation in a manner similar to oligomycin (8,9). Nevertheless, their mode of action, especially of triphenyltins, is still nebulous and inhibition at some other site seems possible (7,9,11).

The availability of *Cercospora beticola* Sacc. strains resistant to triphenyltins (4,5) prompted this study. Our objective was to compare in vitro growth of triphenyltin-sensitive and -resistant strains in the presence of oligomycin to determine whether evolution of triphenyltin resistance has concurrently led to oligomycin resistance. Such cross-resistance would allow useful assumptions regarding the mechanism of triphenyltin action.

MATERIALS AND METHODS

Chemicals. The fungicides used were technical grades of triphenyltin acetate (TPTA) and carbendazim. Oligomycin, a mixture of oligomycin A (15%) and B (85%) (Calbiochem A.G., Lucerne, Switzerland), was provided by S. G. Georgopoulos, Athens College of Agricultural Sciences. Stock solutions were made in distilled water for TPTA and carbendazim and in methanol for oligomycin.

Fungal strains. The *C. beticola* strains were single-spore isolates obtained from naturally infected sugar beets in northern Greece (4). To ascertain homogeneity, each isolate was cultured several times by transferring small mycelial blocks on potato-dextrose agar (PDA), with or without fungicides. The isolates were then maintained on PDA.

In vitro experiments. To compare the growth response of TPTA-sensitive and -resistant strains to oligomycin, the isolates were grown on PDA containing TPTA or oligomycin at various concentrations; the medium was inoculated with mycelial disks (2 mm in diameter) taken from the periphery of 6-day-old colonies grown on PDA. Cultures were then incubated at 25–27 C in the dark. Growth of colonies was expressed in terms of diameter expansion.

RESULTS

Based on their sensitivity to triphenyltins and benzimidazoles, three types of strains could be isolated from the natural population of *C. beticola* in northern Greece—isolates sensitive to both groups of chemicals, those resistant to both groups, and those resistant to benzimidazoles but sensitive to triphenyltins (Table 1). Strains resistant to triphenyltins but sensitive to benzimidazoles could not be found. Determination of the relative abundance of these strains was beyond the scope of this study. We observed, however, that frequency of occurrence of each strain varied greatly with location and was presumably related to the history of fungicide use in each location.

Triphenyltin-sensitive strains, regardless of sensitivity or resistance to benzimidazoles, did not grow on PDA containing 0.125 μg/ml oligomycin (Table 1). Triphenyltin-resistant strains, on the other hand, grew readily on this medium. These results indicate the occurrence of oligomycin-resistant strains in Greece and demonstrate a positive correlation between triphenyltin resistance and oligomycin resistance. Oligomycin resistance is not correlated

Table 1. Growth behavior of 18 *Cercospora beticola* strains on potato-dextrose agar containing triphenyltin acetate (TPTA), carbendazim, or oligomycin[a]

Strain	TPTA (0.5 μg/ml)	Carbendazim (0.1 μg/ml)	Oligomycin (0.125 μg/ml)
7	S	S	S
10	S	S	S
508	S	S	S
514	S	S	S
561	S	S	S
715	S	R	S
507	S	R	S
713	S	R	S
559	S	R	S
557	S	R	S
56	R	R	R
58	R	R	R
92	R	R	R
102	R	R	R
105	R	R	R
88	R	R	R
203	R	R	R
204	R	R	R

[a]S (sensitive) = complete or nearly complete inhibition of growth; R (resistant) = slight inhibition of growth (see also [4]).

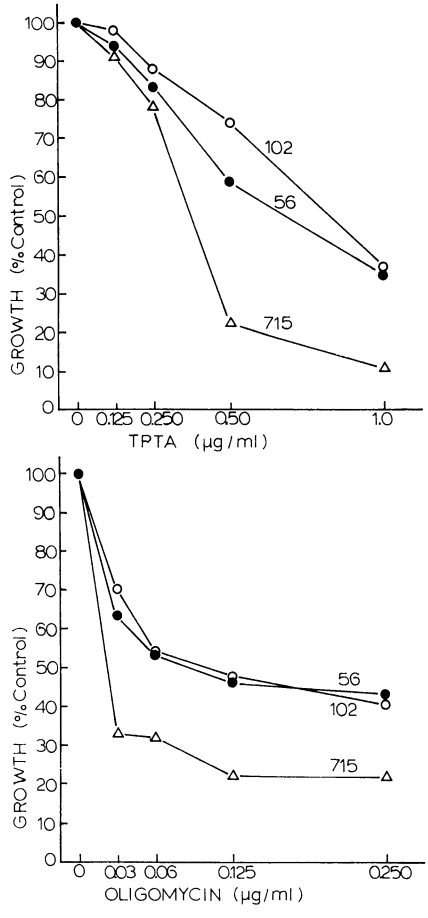

Fig. 1. Growth response of three *Cercospora beticola* isolates (56, 102, and 715) to various concentrations of triphenyltin acetate (TPTA) (top) or oligomycin (bottom). Growth was determined by measuring diameter expansion after inoculation of potato-dextrose agar with mycelial disks (2 mm in diameter) and incubation at 25–27 C for 2 days. Each value is the average of three replicates.

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with benzimidazole resistance.

Positive correlation between triphenyltin resistance and oligomycin resistance of *C. beticola* was also suggested by the dose-response curves obtained with three TPTA-resistant strains with different levels of resistance to TPTA. Strains 56 and 102, which exhibited the most resistance to TPTA, gave identical dose-response curves with oligomycin (Fig. 1). Strain 715, the least resistant to TPTA, was also the least resistant to oligomycin.

The dose-response curves also indicated that the maximum inhibition of growth of the resistant strains is less with oligomycin than with TPTA. Complete inhibition of growth could always be obtained with TPTA but never with oligomycin.

DISCUSSION

The results from this in vitro study may help in understanding the nature of triphenyltin resistance and allow some assumptions on the mode of fungicidal action of triphenyltins. Field development of triphenyltin resistance in *C. beticola* in Greece has been attributed to the selection pressure arising from continuous use of triphenyltin fungicides (4). The mutation(s) conferring resistance to triphenyltins must also be responsible for resistance to oligomycin, because the two types of resistance are associated. Independent mutation(s) is (are) apparently responsible for benzimidazole resistance, because no relationship exists between resistance to benzimidazoles and resistance to triphenyltins (or oligomycin).

The observed association between resistance to TPTA and to oligomycin, structurally unrelated chemicals, supports the hypothesis that triphenyltins have an action similar to that of oligomycin on the ATPase complex of oxidative phosphorylation (7,9), which is not considered the site of action for benzimidazoles (1,2,6). This suggests not only that site modification is the mechanism of resistance, but also that the triphenyltin resistance observed in the field is genetic in nature.

It has been proposed that trisubstituted tin fungicides may also have other effects besides the oligomycin-like inhibition of oxidative phosphorylation (7,9,11). Our observation of higher maximum inhibition by TPTA than by oligomycin suggests that TPTA affects other processes besides oxidative phosphorylation at higher concentrations or that it inhibits oxidative phosphorylation more effectively than oligomycin in *C. beticola.*

Existence of variable degrees of fungal resistance to a chemical is considered to be the expression of a more complex mutational change and/or genetic interaction (3,10). In the case of *C. beticola*, levels of triphenyltin resistance vary quite widely, in contrast to levels of benzimidazole resistance. The possible interference of triphenyltins at some other site(s) besides mitochondrial systems suggests that rather complex genetic changes affect resistance, leading to greater diversity of resistance levels.

This complexity, along with the reduced competitive ability of the resistant strains (5), could explain why triphenyltin resistance took several years to develop. Field sampling for this study did not yield the highly resistant strains that were abundant in 1977 (4), which indicates that highly resistant strains tend to disappear after 2 yr of partial substitution of other fungicides for triphenyltins, as we predicted (5).

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