# Biology of Triphenyltin-Resistant Strains of *Cercospora beticola* from Sugar Beet

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**ABSTRACT**

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Strains of *Cercospora beticola* resistant to triphenyltins in vitro isolated from sugar beets in northern Greece caused leaf spot disease that was not controlled in growth chamber studies by recommended rates of triphenyltin acetate. Resistant strains do not differ from sensitive strains in in vitro growth rate or colony characteristics. No relationship exists between triphenyltin resistance and benzimidazole resistance in the pathogen. Virulence varies among both triphenyltin-resistant and -sensitive strains. Triphenyltin-resistant strains, however, seem to have less competitive ability than triphenyltin-sensitive strains, which could account for the delayed appearance of resistance to this fungicide. Because of their reduced competitive ability, resistant populations in the field may decline after the selective agent (triphenyltins) has been removed.

Additional key word: maneb

Reports of field development of fungal resistance to organometallic fungicides have been rare. Strains of *Cercospora beticola*, the causal fungus of leaf spot disease on sugar beets, resistant to triphenyltin fungicides are an example of acquired resistance that has developed because of the continuous use of these fungicides (2). Population studies revealed that the frequency of occurrence of resistant strains was correlated with the number of years triphenyltins had been used. More information was needed to substantiate these conclusions and to predict the behavior of resistant strains in relation to the wild type under field conditions. This study attempts to compare triphenyltin-sensitive and -resistant strains with respect to basic characteristics of biology and pathogenicity. A similar study of benomyl-sensitive and -resistant strains of the same fungus has been published (5).

## MATERIALS AND METHODS

**Fungal strains.** The *C. beticola* strains were single-spore isolates maintained on sugar beets in a growth chamber. The strains were isolated as sensitive (S) or resistant (R) to triphenyltins from sugar beets in northern Greece according to previously described procedures (2). Both groups of strains were grown on water agar for 5 days and then on tomato juice agar for 10 days before being transferred to leaves.

**In vitro experiments.** To learn whether R and S strains differ in growth on an artificial medium and whether resistance to triphenyltins is correlated with resistance to benzimidazoles, conidia of both strains were transferred from sugar beet leaves into petri dishes containing fungicide-free *Aspergillus* Complete Medium (ACM) (4). After 6 days, mycelium on agar blocks (20 $mm^2$) was transferred onto the same medium amended with various concentrations of technical grades of triphenyltin acetate (TPTA), triphenyltin chloride (TPTC), carbendazim, and maneb. The cultures were incubated at 22–25 C, and increases in colony diameters were measured.

**In vivo experiments.** These experiments were performed to 1) confirm field observations that triphenyltin-R strains cause disease that cannot be controlled with recommended rates of TPTA, 2) determine whether resistance is maintained after consecutive inoculations of unsprayed sugar beets, and 3) compare virulence and competitive ability of R strains with those of the wild type.

Sugar beets (*Beta vulgaris* L.

**Table 1.** Mycelial growth[a] of *Cercospora beticola* isolates grown on *Aspergillus* Complete Medium amended with triphenyltin acetate or triphenyltin chloride

| Strain | Fungicide concentration (μg/ml) 0 | 0.125 | 0.250 | 0.500 | 1.000 |
|---|---|---|---|---|---|
| | **Triphenyltin acetate[b]** | | | | |
| $R_3$ | 18.0 | 15.0 | 13.5 | 11.7 | 11.7 |
| $R_{16}$ | 17.1 | 13.2 | 11.7 | 10.2 | 10.0 |
| $R_{19}$ | 17.2 | 15.7 | 13.3 | 12.0 | 11.0 |
| $S_5$ | 17.5 | 3.3 | 0 | 0 | 0 |
| $S_{12}$ | 16.2 | 14.0 | 7.0 | 2.3 | 0 |
| $S_{16}$ | 16.5 | 3.7 | 0 | 0 | 0 |
| | **Triphenyltin chloride[b]** | | | | |
| $R_3$ | 18.7 | 17.5 | 14.2 | 12.3 | 11.1 |
| $R_{16}$ | 17.0 | 15.3 | 12.7 | 9.8 | 8.7 |
| $R_{19}$ | 18.8 | 6.2 | 14.4 | 12.0 | 10.2 |
| $S_5$ | 16.2 | 2.3 | 0 | 0 | 0 |
| $S_{12}$ | 15.7 | 13.3 | 9.5 | 1.7 | 0 |
| $S_{16}$ | 16.5 | 4.3 | 0 | 0 | 0 |

[a] Colony diameter (mm), measured 6 days after inoculation with agar block (20 $mm^2$) bearing mycelium 6 days old. Each value is the average of three replications.
[b] Technical grades of fungicides of a minimum 99% purity.

**Table 2.** Mycelial growth[a] of *Cercospora beticola* isolates on *Aspergillus* Complete Medium amended with carbendazim and/or maneb

| Strain | Fungicide[b] Check | Carbendazim (μg/ml) 0.1 | 1.0 | 10.0 | Maneb (μg/ml) 4 | 8 | 16 | Carbendazim + maneb (μg/ml) 0.1 + 2 |
|---|---|---|---|---|---|---|---|---|
| $R_3$ | 19.3 | 6.2 | 0 | 0 | 16.5 | 13.5 | 8.0 | 4.8 |
| $R_{16}$ | 18.1 | 16.6 | 16.3 | 4.2 | 14. 3 | 11.3 | 8.3 | 15.8 |
| $R_{19}$ | 18.6 | 8.4 | 0 | 0 | 16.3 | 13.2 | 10.6 | 6.8 |
| $S_5$ | 18.5 | 7.3 | 0 | 0 | 15.6 | 14.3 | 10.1 | 4.9 |
| $S_{12}$ | 20.0 | 15.5 | 14.7 | 1.2 | 16.5 | 12.8 | 10.8 | 13.5 |
| $S_{16}$ | 18.0 | 5.0 | 0 | 0 | 15.0 | 13.6 | 9.6 | 4.2 |

[a] Colony diameter (mm), measured 6 days after inoculation with agar block (20 $mm^2$) bearing mycelium 6 days old. Each value is the average of three replications.
[b] Technical grades of carbendazim and maneb of 99 and 81.7% purity, respectively.

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'Kawemono') were grown in pots 18 cm in diameter, three plants per pot, in a growth chamber at 25 C with a 12-hr photoperiod. Inoculum was produced by transferring conidia of the various strains from stock leaves onto tomato juice agar medium and allowing the colonies to sporulate under fluorescent light for 10 days. The colonies were then rinsed with distilled water to detach the conidia. Sugar beets (six to eight leaves) in each pot were sprayed with 10 ml of the conidial suspension ($8 \times 10^5$ conidia/ml). Each pot was enclosed in a plastic bag for 24 hr; the bags were opened for 6 hr/day during the next 5 days. Bags were then removed, and the numbers of spots per plant were recorded at various time intervals. Fungicide-treated sugar beets were sprayed with a 60% wettable powder formulation of TPTA (0.3 kg a.i./ha) 1 day before inoculation.

To determine whether R strains could cause disease that is not controlled by TPTA, three pots of TPTA-treated sugar beets and three pots of untreated sugar beets were inoculated with an R or an S strain, and disease development was observed. The experiment was repeated twice, once with strains $R_3$ and $S_5$ and once with strains $R_{19}$ and $S_5$. Since the results were similar, the data of only one experiment ($R_3S_5$) are presented.

To examine retention of resistance, three R strains ($R_3$, $R_{16}$, and $R_{19}$) and one S strain ($S_5$) were transferred four consecutive times to untreated sugar beets. In these experiments, conidia from leaves of each preceding transfer were used as inoculum. Conidial isolates of strains from the original stock leaves and from leaves bearing conidia of the fourth transfer were compared for growth on ACM with or without TPTA.

Virulence and competitive ability of the strains were examined by inoculating three plants in each of four pots of untreated sugar beets with conidia of one of four strains ($S_{16}$, $S_5$, $R_{19}$, and $R_{16}$). Two other pots were inoculated with a 1:1 mixture of conidia from two strains, one with $S_{16}$ + $R_{19}$ and the other with $S_5$ + $R_{19}$. Leaf spots were counted 12 days after inoculation. From plants of each pot, 100 monospore isolates were randomly obtained on ACM. Percentage of resistant isolates was then determined by transferring agar blocks bearing mycelium to ACM containing 0.25 μg/ml TPTA. For the mixed inoculations, the percentages were further confirmed by noting the color of colonies on fungicide-free ACM; colonies of $R_{19}$ were slightly lighter in color than colonies of $S_{16}$ and $S_5$.

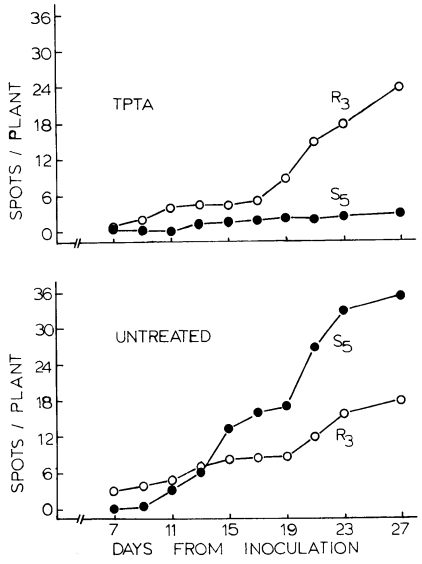


**Fig. 1.** Severity of leaf spot disease of triphenyltin acetate (TPTA)-treated (0.3 kg a.i./ha) sugar beets (top) and untreated sugar beets (bottom) after inoculation with a triphenyltin-sensitive ($S_5$) or -resistant ($R_3$) strain of *Cercospora beticola*.

## RESULTS AND DISCUSSION

**In vitro experiments.** Growth on fungicide-free medium varied within but not between groups of strains. S isolates increased in diameter an average 6.5 mm with a range of 4.9–8.3 mm between 4 and 7 days after inoculation. Similarly, R strains increased an average 6.7 mm with a range of 5.7–8.0 mm. Changes associated with evolution of resistance, therefore, do not seem to affect the control mechanisms of in vitro growth of the pathogen. Color of colonies varied somewhat but did not appear to be related to triphenyltin resistance.

TPTA and TPTC in the medium at concentrations as low as 0.125 μg/ml inhibited growth of the S strains (Table 1). Growth of the R strains was inhibited only by relatively high fungicide concentrations. Intermediate levels of resistance were also evident ($S_{12}$). Strains grew similarly on media amended with either TPTA or TPTC, indicating positive cross-resistance to these fungicides in agreement with a previous study of the pathogen (2).

Growth data for the strains on ACM amended with carbendazim and/or maneb are shown in Table 2. Some strains were resistant to carbendazim, indicating that resistance to benzimidazoles was retained despite discontinuance of their use in 1973, as predicted by Dovas et al (1). As expected, none of the strains exhibited resistance to maneb. The fact that a benzimidazole-R strain was found among the triphenyltin-R strains ($R_{16}$) as well as among the triphenyltin-S strains ($S_{12}$) leads to the conclusion that resistances to these two groups of fungicides are not related and suggests that the genetic or biochemical mechanisms of resistance to these fungicides differ. Therefore, strains resistant to triphenyltins and to benzimidazoles may have developed concurrently but independently.

**Table 3.** Virulence and competitive ability of *Cercospora beticola* strains on untreated 'Kawemono' sugar beets under growth chamber conditions[a]

| Strain | Strain virulence (no. of lesions)[b] | % Recovered R | % Recovered S |
|---|---|---|---|
| $S_{16}$ | 356 | 0 | 100 |
| $S_5$ | 2,262 | 0 | 100 |
| $R_{19}$ | 1,335 | 100 | 0 |
| $R_{16}$ | 495 | 100 | 0 |
| $S_{16}$ + $R_{19}$ | ··· | 33[c] | 67 |
| $S_5$ + $R_{19}$ | ··· | 12 | 88 |

[a] Temperature 25 C, photoperiod 12 hr. Plants were enclosed in plastic bags for the first 24 hr after inoculation and for 18 hr a day for the next 5 days.

[b] Average number of lesions counted on three plants per replicate 12 days after inoculation by spraying with 10 ml of conidial suspension ($8 \times 10^5$ conidia/ml).

[c] Strain $R_{19}$ also differentiated by color of mycelium.

**In vivo experiments.** A previous study (2) established that resistance is maintained after several in vitro transfers of R isolates. In this study, three R strains were consecutively passed four times through unsprayed sugar beet seedlings. These in vivo transfers did not alter colony characteristics or tolerance to TPTA, providing additional evidence that resistance resulted from permanent genetic or physiologic changes in the pathogen rather than temporary adaptation.

When untreated sugar beets were inoculated with conidia from either S or R strains, leaf spot disease developed (Fig. 1 and Table 3). By contrast, only R strains caused an appreciable amount of disease on TPTA-treated sugar beets (Fig. 1). These findings provide further evidence that triphenyltins failed to control the disease in the field because R strains were present.

S and R strains both varied in infection potential (Table 3), suggesting that virulence is independent of resistance to triphenyltins. The competition between S and R strains, however, seems to favor the former, based on percentage recovery. When sugar beets were inoculated with a 1:1 mixture of conidia from S and R strains, S strains were isolated from the resulting leaf spots more often than R strains (Table 3). The reduced competitive ability of the R strains observed in the in vivo tests under growth chamber conditions leads to the prediction that the R population in the field may decline when the use of triphenyltin fungicides is discontinued.

Reduced competitive ability of the R strains may also partly account for the delayed appearance (after about 10 years of use) of fungal resistance to triphenyltins in the field. Another reason may be the postulated nonspecific mode of action for these compounds (3), since it is generally accepted that resistance to multisite