The Cell-Wall Phosphatase of Cotton (*Gossypium*) is Inhibited by Kelthane

By LARRY S. DALEY,* PATRICIA CARROLL† and HARRY MUSSELL†

*Department of Biological Sciences, North Texas State University, Denton, TX 76203, U.S.A., and

†Boyce Thompson Institute, Cornell University, Ithaca, NY 14853, U.S.A.

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Kelthane [4,4'-dichloro- α -(trichloromethyl)benzhydrol] was previously shown to decrease the limited tolerance of susceptible varieties of cotton (*Gossypium*) to *Verticillium* wilt. Kelthane was shown in the present study to inhibit the cell-wall *p*-nitrophenyl phosphatase of cotton. In view of information already establishing the cell wall as a primary site of action of *Verticillium* wilt, the data are interpreted as suggesting an as yet undefined interaction between Kelthane, cell-wall phosphatase and *Verticillium*-resistance mechanisms of the cell wall.

Kelthane [4,4'-dichloro- α -(trichloromethyl)benzhydrol] is a frequently used miticide. Kelthane is very closely related in structure to DDT [1,1'-(2,2,2-trichloroethylidene)bis(4-chlorobenzene)]. DDT and Kelthane differ only in one substituent (Fig. 1). When Kelthane is applied to cotton (*Gossypium*) plants, it increases the degree of sensitivity of susceptible varieties (i.e. *G. hirsutum* var. M-8) to *Verticillium* wilt caused by *Verticillium albo-atrum* Reinke and Bert (Mussell & Ramftl, 1972). No change in reaction to *Verticillium* is observed when the *Verticillium*-tolerant varieties (i.e. *G. barbadense* var. Seabrook–Sea Island) are infected (Mussell & Ramftl, 1972). The metabolism of Kelthane in plants is relatively unknown (Scheel & Sandermann, 1977). However, other chlorinated compounds have similar effects (Nilsson, 1977).

The plant cell wall is usually considered a primary site of resistance to fungal pathogens. Proteins and enzymes associated with plant cell walls are apparently involved in normal plant growth responses and participate in host–parasite interactions during pathogenesis (Mussell & Strand, 1977). *p*-Nitrophenyl phosphatase, having at least some of the characteristics associated with ATPase, has been found on the cell walls of the cotton cultivars described above (this is the first of a series of papers describing various aspects of activity). Since other chlorinated hydro-

carbons, including DDT, have been reported to inhibit ATPase (Matsumura, 1978; Weinbach, 1957), we tested Kelthane and found it to inhibit the *p*-nitrophenyl phosphatase activities of the cell walls of cotton.

Experimental

Cell-wall preparations

Cell walls were prepared by the method of Barnett (1974). The cell-wall suspension was diluted to half strength with glycerol and stored at -20°C until used. Storage did not decrease phosphatase activity.

Reaction conditions

The assay utilizes the substrate *p*-nitrophenyl phosphate dicyclohexylammonium salt, which was stored frozen as 25 mM solution until used. The standard buffer was 0.2 M-Tricine {*N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine} titrated to pH 8.1 (at room temperature) with 0.2 M-Tris. No inorganic salts were included unless specified.

The temperature of the assay was 30°C , and 8 mM-*p*-nitrophenyl phosphate was saturating under all conditions tested, at the standard assay conditions [substrate/buffer/cell-wall suspension (1:1:1, by vol.)]. The assay was linear with cell-wall concentration, and with time for about 10 min. Boiled cell-wall controls had no activity. The rate was determined by increase in A_{405} . The millimolar absorption coefficient (ϵ) used was $18.5 \text{ mmol}^{-1} \cdot \text{cm}^{-1}$. ATPase was assayed by a coupled reaction using lactate, dehydrogenase and pyruvate kinase (Daley & Vines, 1977). The technical-grade Kelthane preparation was solubilized in a minimal amount of ethanol, and the purified crystalline Kelthane in glycerol. Controls included equal amounts of the appropriate solvent. Cell-wall preparations used to assay effects of

Fig. 1. Structures of Kelthane and DDT
 For Kelthane, R = OH; for DDT, R = H.

technical-grade Kelthane were fresh, and those used with crystalline Kelthane were stored at -20°C in glycerol as described.

Results and Discussion

Plant cell-wall enzymes have been reported with some frequency (Lund & Mapson, 1970; Lampert & Northcote, 1960; Hall & Butt, 1968). These enzymes were first thought to be artefacts of poorly prepared cell walls; however, with better cell-wall preparations, these enzymes continue to demonstrate their activity (Yung & Northcote, 1975; Gross, 1977; Gross & Janse, 1977; Barnett, 1974). Among these enzymes are some that are implicated in defence mechanisms against pathogenic fungi (Mussell & Strand, 1977). Cell-wall phosphatases have been reported (Wainwright & Woolhouse, 1978), and their activity may be a reflection of the presence of ATPase associated with the cell wall (Hooper *et al.*, 1978; Hall & Butt, 1969; Hall, 1971; Al-Azzawi & Hall, 1977). If these phosphatases are truly ATPases, as are those of Albers & Koval (1972), then they are of considerable interest to those working with transport phenomena (Hodges, 1976), and to those who find evidence that these enzymes may be the primary site of attack by the pathogen's metabolites (Phillips *et al.*, 1978; Steele *et al.*, 1978; Mondal, 1978; Selman & Durbin, 1978). This is because ATPases are considered to be part of a critical support and supply system of the plant cells (Hodges, 1976).

When Kelthane is applied to *V. albo-atrum*-infected cotton plants, susceptible varieties (M-8) become extremely sensitive to the pathogen, whereas tolerant varieties, Seabrook-Sea Island, retain their tolerant characteristics (Mussell & Ramftl, 1972).

Technical-grade Kelthane inhibits *p*-nitrophenyl phosphatase and ATPase activity of both tolerant (Table 1) and susceptible varieties (Fig. 2). Fig. 3 shows similar information obtained by using crystalline Kelthane (98%). Both susceptible and tolerant sources of cell walls have phosphatase activity that is inhibited by Kelthane. The K_i is in the micromolar range and is therefore taken as an indication of strong interaction of the inhibitor with the enzymic activity in question. Crystalline Kelthane (kindly

given by Rohm and Haas Co., Philadelphia 5, PA, U.S.A.) was about 98% pure. Thus an extremely active impurity with a K_i in the nanomolar range would be required if it were to account for the

Fig. 2. Dixon plot of inhibition of cotton (var. M-8) cell-wall phosphatase by technical-grade Kelthane. Substrate concentrations were: $\circ$, 3.3; $\bullet$, 5.0; and $\square$, 6.7 μM . Abbreviation used: est., estimated.

Fig. 3. Inhibition of cotton cell-wall phosphatase by crystalline Kelthane

$\circ$, *G. hirsutum* var. M-8; $\bullet$, *G. barbadense* var. Seabrook-Sea Island. M-8 and Seabrook-Sea Island are varieties of cotton susceptible and tolerant respectively to *Verticillium* wilt.

Table 1. Effect of Kelthane on the ATPase activity of cotton (*G. barbadense* var. Seabrook-Sea Island) cell walls

Treatment	Rate (μmol of P_i /h per g of cell walls)	Inhibition (%)
None	150.0	—
Boiled	0	100
15 μM -Kelthane	84.4	43

inhibitory effect. Given the rapidity of the assays, metabolite production from Kelthane (Scheel & Sandermann, 1977) is unlikely but possible, and may have influenced the results.

One interpretation of our results is that the cell-wall *p*-nitrophenyl phosphatase is not associated with the weak tolerance of the M-8 variety of cotton to *Verticillium*. In view of this, the inhibition of phosphatase is an effect independent from the increase in susceptibility to *Verticillium* that follows Kelthane application. However, since the cell-wall phosphatases are believed to be part of the plant's transport complex (Hodges, 1976), and the wilt symptoms of the disease are indicative of disruption of these transport activities (Dimond, 1970; Basham & Bateman, 1975), it seems reasonable to consider a common linkage between the two Kelthane effects: the loss of M-8 variety's weak tolerance to *Verticillium* wilt and the inhibition of the cell-wall phosphatase. Yet it is premature to consider the matter further until more information on the system is obtained.

We note that two applications of this information may be suggested: (a) when Kelthane is applied to *Verticillium*-susceptible varieties of tomato and cotton, fungicides should be applied at the same time; and (b) Kelthane may serve as yet another inhibitor to characterize phosphatases and ATPases.

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