

Pesticide Effects on the Plant Cuticle: I. Response of *Brassica oleracea* L. to EPTC as Indexed by Epicuticular Wax Production¹

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Abstract. Production of epicuticular wax by expanding leaves of cabbage (*Brassica oleracea* L. var. capitata cv. Market Prize) was inhibited by S-ethyl dipropylthiocarbamate (EPTC) and trichloroacetic acid (TCA). Increasing the concentration of EPTC (0, 0.28, 0.56, 1.12 and 2.24 kg/ha) resulted in greater inhibition of epicuticular wax production. Both soil and foliar application of EPTC were effective. All leaves not fully expanded at time of application were affected, and no regeneration of epicuticular wax was evident after full leaf expansion. The difference between the amount of wax produced by the control and EPTC-treated plants gradually declined on those leaves which developed after EPTC application. This reduction was accompanied by an absence of wax bloom and a reduction of surface wax fine-structure.

Pesticide chemicals can markedly alter cuticle development on expanding leaves (4, 7, 9, 11, 14, 24, 26, 27). Plants with altered cuticles are often more sensitive to their environment, as indexed by increased scorch (8), water loss (1, 17), spray retention, (8, 17), and phytotoxicity to herbicide sprays (8, 11, 17).

All plant parts exposed to the environment are covered with a cuticle, a thin continuous, nonuniform noncellular lipoidal membrane. Generally, it consists of a cutin matrix with epicuticular (surface) wax deposited on the outer surface and cuticular wax embedded in the matrix (15, 20, 22). It protects the plant against the external environment (10) and serves as the main barrier which a compound must transverse before penetration and cellular uptake can occur (5). Waxes associated with the cuticle impede penetration of foliar applied chemicals into leaves (2, 3, 6, 16) and influence cuticular transpiration (1, 13, 18, 19). Physical removal of the epicuticular waxes by brushing leaf surfaces, or by solvent extraction from isolated cuticles greatly increases penetration and water loss (2, 3, 6, 16). Therefore, any modification of cuticular structure by physical, chemical or environmental factors may affect its permeability and hence its efficiency as a protective covering.

Greater plant sensitivity to pesticide chemicals or environmental stress has been associated with members of 2 groups of compounds, the carbamates (11, 24, 26, 27), and the short chained chlorinated fatty acids (8, 9, 14, 17). Although these responses have been associated with less epicuticular wax (11, 17), the dynamics of plant response and effects on other cuticular constituents is not known.

We have initiated a study designed to characterize the effect of pesticide chemicals on development of the cuticle and to relate these findings to cuticular permeability. In this first paper we report on the dynamics of plant response, using epicuticular wax as an index to a given chemical treatment, and describe the optimum chemical and plant conditions needed to produce a desired response to provide a basis for further studies.

Materials and Methods

Plant culture. Cabbage was selected as the experimental plant material because of its conspicuous wax bloom, fast growth, and

uniform leaf characteristics. Seeds were germinated in vermiculite and when seedlings were in the 2 to 4 leaf stage, they were transplanted into 10-cm diam peat pots. The plants were grown in a greenhouse at a minimum temp of 20°C and a 14-hr light period. Water was provided for each pot by plastic irrigators to avoid wetting the leaves. Fertilizer (20-20-20) was supplied through the watering system twice monthly. In an effort to keep pesticide residues on leaf surfaces at a minimum, insects were controlled by fumigation (1,2-dibromo-2,2-dichloroethyl dimethylphosphate, in acetone).

Pesticide application. S-Ethyl dipropylthiocarbamate (EPTC) and trichloroacetic acid (TCA) were applied as aqueous soil drenches (10 ml/pot) when the plants were in the 6-leaf stage. The youngest visible node was noted for each plant at time of treatment. In all cases the 7th node was in the bud stage at time of application and would be the next leaf to emerge. Lower and higher node numbers represent older and younger leaves, respectively. A commercial emulsifiable concentrate (75%) was used and rates were expressed in kg/ha of active chemical. Inert ingredients of commercial EPTC emulsifiable concn did not affect wax deposition (11).

Epicuticular wax and leaf area determination. Designated leaves were harvested carefully, allowed to wilt slightly to assure stomatal closure, and each was dipped for 10 sec in each of 4 successive 200 ml portions of redistilled chloroform. The washings were combined and dried over anhydrous sodium sulfite for 10 min before filtering into tared flasks. Chloroform was removed under reduced pressure on a rotary evaporator at a temp not exceeding 40°C. The flasks were dried to constant wt (40°C) and the wax was determined by subtraction. Leaf area was calculated from the wt of leaf outlines cut from a uniform grade of paper. Data were expressed in μg of wax/cm² leaf area. Three determinations were made for each treatment (each consisted of 3 to 5 leaves).

Photomicrographs. Sections (1 cm²) from leaves of control and EPTC-treated plants were mounted on glass slides with double sticky tape. Photomicrographs were taken with a Wild M-20 research microscope equipped with a 35 mm film holder, and an incident light attachment for reflected light photography.

Method of application. An equivalent of 2.24 kg/ha EPTC was applied to each plant by one of 3 methods: (a) as a soil drench (75% emulsifiable concentrate in 10 ml of water), (b) a granular formulation (10%) incorporated in the surface 2.5 cm of soil, and (c) a foliar spray consisting of 10 ml of the soil drench solution plus 0.1%, X-77 (alkylaryl polyoxyethylene glycols, free fatty acids, and isopropanol), 35% spray retention was attained.

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²Baker, E. A. The effect of environmental factors on the development of leaf wax of *Brassica oleracea* var. Gemnifera. MSc Thesis, Univ. Bristol, 1972. 134 pp.

Duration of response on a given node, and on developing nodes. Plants were grown according to the general method in 15-cm diam clay pots. EPTC was applied as a soil drench (2.24 kg/ha). Duration of response for a given leaf was indexed by measuring the amount of epicuticular wax present on the leaf produced at the 7th node 10, 20, 40, and 50 days after treatment. Duration of response for nodes developing after application was determined by measuring the amount of epicuticular wax present on each leaf developing on the 5th and succeeding nodes 10, and 50 days after treatment, *i.e.* wax quantities for treated and control plants were compared on leaves of similar physiological development.

Results

EPTC and TCA. Both compounds inhibited epicuticular wax formation on leaves developing for 14 days subsequent to treatment (Table 1). No phytotoxicity or morphological abnormalities were observed as a result of treatment.

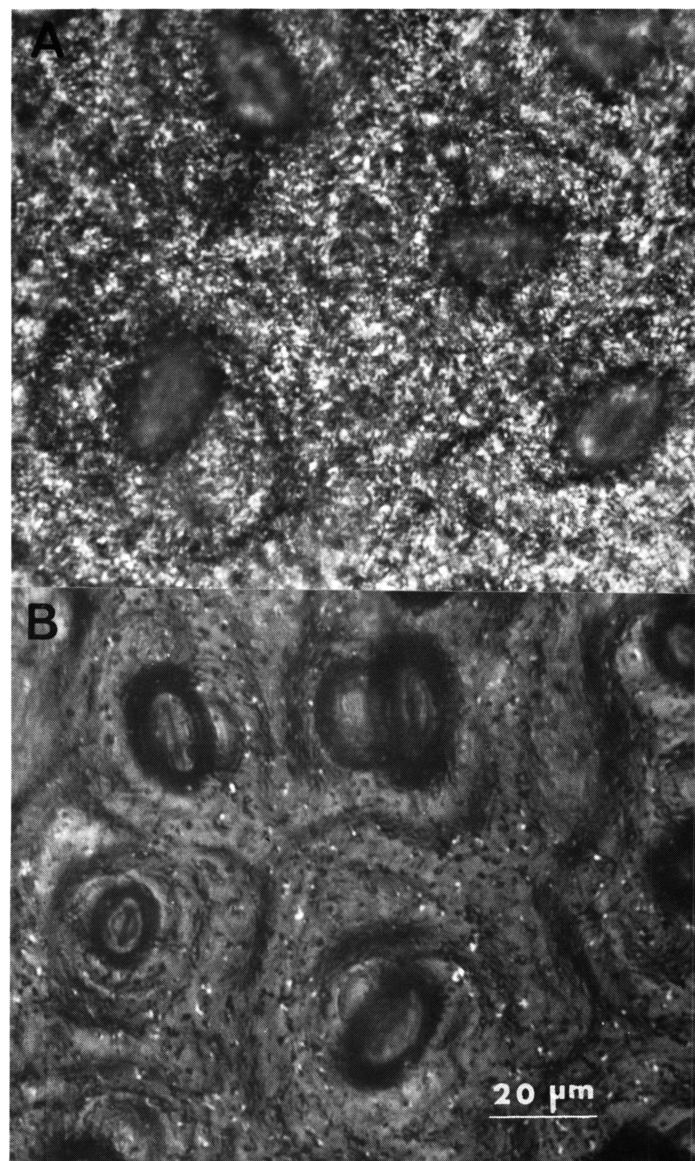


Fig. 1. Photomicrographs illustrating the epicuticular wax on the upper surface of cabbage leaves from control (A) and EPTC-treated (B) plants. EPTC (2.24 kg/ha) was applied as a soil drench when these leaves were in the bud stage and photographed 21 days after treatment.

Table 1. Inhibition of epicuticular wax formation of developing leaves of cabbage.

Measurement	Treatment ^z		
	Control	EPTC (2.2 kg/ha)	TCA (11.2 kg/ha)
Epicuticular wax ($\mu\text{g}/\text{cm}^2$)	67.0a	34.0c	52.0b
Inhibition (%)	0	49.3	22.4

^zMean separation by Tukey's ω test, $P = 0.05$.

Wax bloom. EPTC (2.24 kg/ha) caused a marked reduction in the wax bloom conspicuous on surfaces of developing leaves. This was associated with a reduction in the fine-structure present on the leaf surfaces as apparent by visual observation, and by comparison of reflected light photomicrographs of control and treated plants (Fig. 1).

Method of application. EPTC significantly inhibited epicuticular wax production irrespective of mode of application (Table 2). Both soil treatments, however, resulted in a greater reduction than the foliar spray.

Table 2. Comparative inhibition of epicuticular wax production on developing leaves of cabbage as related to method of EPTC application. All EPTC treatments applied at 2.24 kg active chemical per ha. Retention of foliar spray was approx. 35%.

Measurement	Method of Application ^z			
	Control	Soil drench	Granular, soil incorporated	Foliar spray
Epicuticular wax ($\mu\text{g}/\text{cm}^2$)	35.0a	14.9c	11.2c	21.7b
Inhibition (%)	0.0	57.4	68.0	38.0

^zMean separation by Tukey's ω test, $P = 0.05$.

Concn response. Increasing concn of EPTC resulted in a corresponding increase in inhibition of epicuticular wax production, although degree of inhibition was not proportional to the increase in rate applied (Table 3).

Table 3. The effect of concentration of EPTC (soil drench) on inhibition of epicuticular wax production in developing leaves of cabbage 14 days after treatment.

Measurement	EPTC (kg/ha) ^z				
	0.0	0.28	0.56	1.12	2.24
Epicuticular wax ($\mu\text{g}/\text{cm}^2$)	44.4a	35.0b	29.6c	27.4c	22.8d
Inhibition (%)	00.0	21.2	33.3	38.3	48.6

^zMean separation by Tukey's ω test, $P = 0.05$.

Duration of response - for a given leaf. Approx $22 \mu\text{g}/\text{cm}^2$ of epicuticular wax was present on EPTC-treated leaves (leaves produced at the 7th node) 10 days after treatment compared to $46 \mu\text{g}/\text{cm}^2$ for controls (Fig. 2). During the next 40 days the wax level on leaves produced at the same node (7th) increased to $28 \mu\text{g}/\text{cm}^2$ for EPTC treated and $49 \mu\text{g}/\text{cm}^2$ for nontreated plants. Therefore, neither the magnitude of the differences between EPTC-treated and control nor the absolute wax level changed significantly during the subsequent development of leaves arising from the same node. Although leaves from EPTC-treated plants appeared slightly larger, the difference was not statistically significant (Fig. 2).

Duration of response - for a given plant. Ten days after treatment the difference in epicuticular wax between the control and EPTC-treated plants was approx $20 \mu\text{g}/\text{cm}^2$ for each of the first 5 leaves developing after treatment, *i.e.* leaves from the 5th through the 9th node (Fig. 3). At this time none of the leaves were fully expanded. Fifty days after application, the differences in the amount of epicuticular wax on leaves

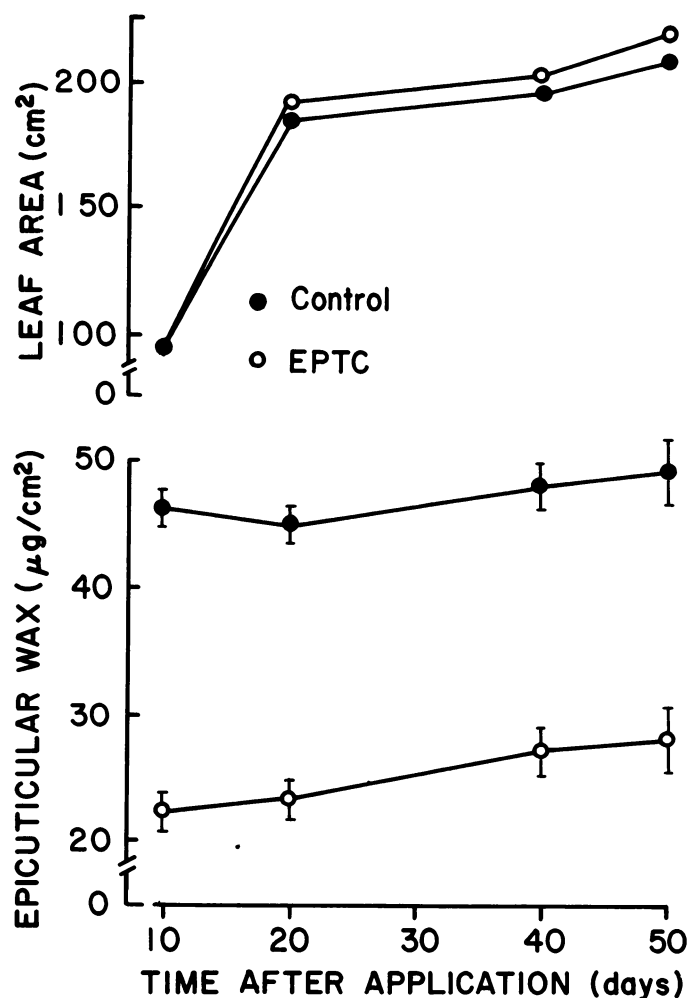


Fig. 2. Effect of EPTC on leaf area and quantity of epicuticular wax on the leaf developing at the 7th node as related to time after application. Vertical brackets denote standard deviation for the mean.

produced on succeeding nodes (10th to 20th node) was correspondingly less. From the 20th to the 25th node no significant difference was apparent between the control and EPTC-treated plants (Fig. 4). Interestingly, less epicuticular wax was present per unit area on apical than on basal leaves of control cabbage plants.

Discussion

Our data confirm that epicuticular wax production can be markedly inhibited on developing leaves of cabbage by soil or foliar treatment with EPTC. We further described the dynamic response of the cabbage plant to EPTC. The maximum reduction in epicuticular wax (approx. 50%) was achieved on leaves developing immediately subsequent to soil treatment. The effect was progressively less on leaves which developed on subsequent nodes.

It is generally accepted that cuticle development continues until the leaf is morphologically mature (23). As demonstrated by Schieferstein and Loomis (21), epicuticular wax is deposited only during leaf expansion. This explains why in our study the magnitude of difference in epicuticular wax between control and EPTC-treated plants remained almost constant when wax levels were followed during progressive development of the leaf arising from the 7th node. This leaf was in the bud stage at time of treatment and attained 90% of full expansion within 20 days, therefore, the period of greatest wax production coincided with the period of maximum exposure to EPTC.

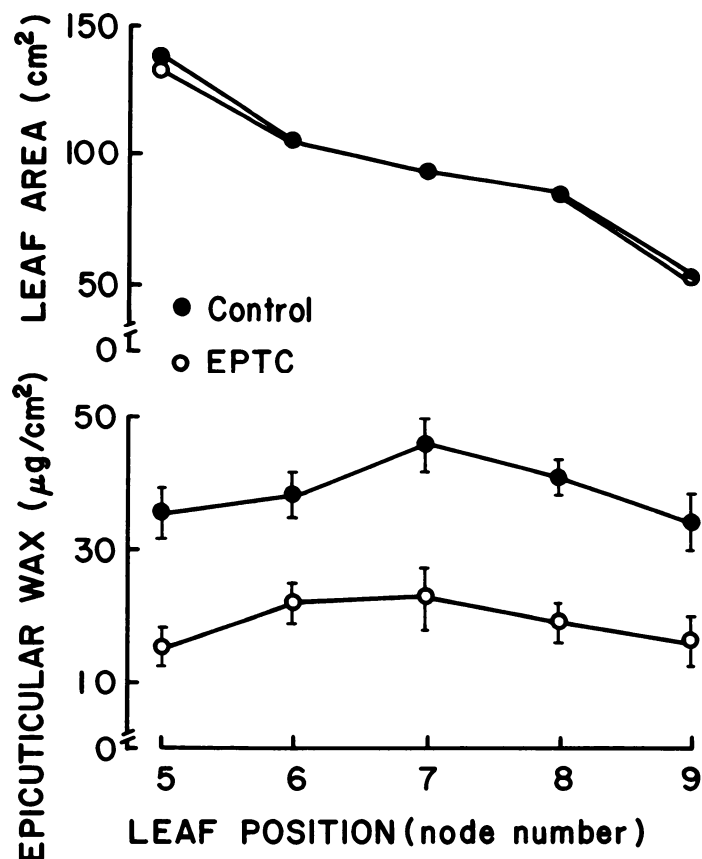


Fig. 3. Effect of EPTC on leaf area and quantity of epicuticular wax on leaves of different physiological age 10 days after treatment. Plants were in the 6th leaf stage at time of treatment. Vertical brackets denote standard deviation for the mean.

The decreasing effect of EPTC on leaves developing from subsequent nodes is undoubtedly related to the persistence of this chemical in the soil. EPTC is a highly volatile compound and its persistence in soil depends on several factors (12). A loss in activity below some critical threshold value would permit the

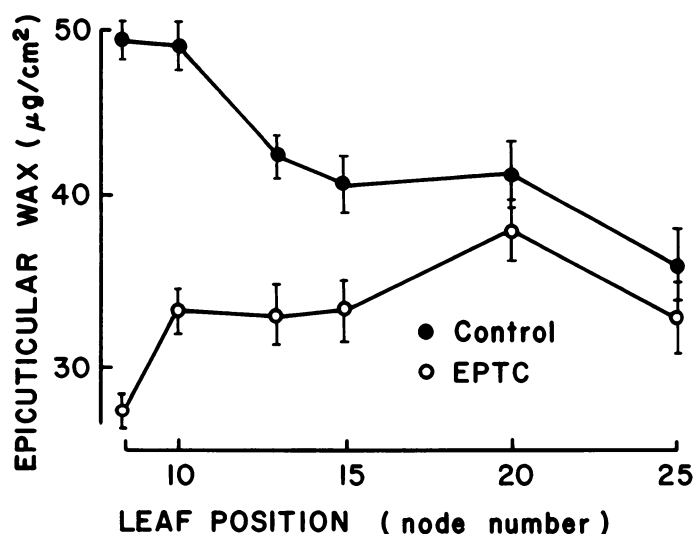


Fig. 4. Effect of EPTC on quantity of epicuticular wax on leaves of different physiological age 50 days after treatment. Plants were in the 6th leaf stage at time of treatment. Vertical brackets denote standard deviation for the mean.

subsequently developing tissue to escape the EPTC effect. Our study indicates that rates as low as 0.28 kg/ha can result in an appreciable inhibition of wax development (Table 3).

Gentner (11) found that only those leaves which were in the bud stage at time of treatment were affected. However, we found that in addition to leaves in the bud stage those present but not fully expanded were also affected. Fluctuations in absolute quantities of epicuticular wax for EPTC and control plants followed the same general pattern (Fig. 4). Whether this effect is an artifact, or is due to uncontrolled changes in the environment has not been determined. It is known that the environment during leaf expansion can influence the quantity and fine-structure of the epicuticular wax² (25).

In addition to a reduction in epicuticular wax production, EPTC caused changes in surface fine-structure (Fig. 1). Juniper (14) correlated TCA treatment with an alteration of surface fine-structure and an increase in wettability of pea leaf surfaces. He concluded that such alteration increased susceptibility to herbicidal sprays. Increased wettability could indirectly influence penetration of foliar applied compounds by increasing the contact area between the chemical spray and leaf surface.

It is clear that EPTC and TCA inhibit epicuticular wax production and modify the fine-structure on the leaf surface. Unfortunately, little is known about chemical or structural changes which may be induced in other cuticular components. Increased sensitivity of EPTC- and TCA-treated plants to subsequently applied herbicidal sprays (8, 11, 17) suggests increased penetration, and recently Davis and Dusbabek (7) have been able to demonstrate increased uptake of labeled pesticides by peas exposed to another thiocarbamate, diallate [S-(2,3-dichloroallyl)diisopropylthiocarbamate]. The data reported here provide a basis for further study of pesticide effects on the cuticle which may improve our understanding of cuticular permeability.

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