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Plant Epicuticular Lipids:

ALTERATION BY HERBICIDAL CARBAMATES[1]

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GERALD G. STILL, DAVID G. DAVIS, AND G. L. ZANDER[2]
Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Metabolism and Radiation Research Laboratory, State University Station, Fargo, North Dakota 58102

ABSTRACT

The effect of several carbamates and trichloroacetic acid on the biosynthesis of epicuticular lipids from leaves of pea (*Pisum sativum*) was tested by chemical and visual methods. The carbamates tested included *S*-(2,3-dichloroallyl)diisopropylthiocarbamate (diallate), *N*-(3-chlorophenyl)isopropylcarbamate (chloropropham), *S*-ethyl dipropylthiocarbamate, and 2-chloroallyl diethyldithiocarbamate. Diallate reduced epicuticular lipids by 50% when the plants were root-treated and by 80% when vapor-treated. These results were supported by scanning electron microscopy and carbon replica techniques with transmission electron microscopy. The ratio of wax lipid components in the diallate-treated plants remained unchanged, with the exception of the primary alcohols, which were reduced. Diallate appears to interfere with the biosynthesis of a precursor to the elongation-decarboxylation pathway of lipid synthesis. *N*-(3-Chlorophenyl)isopropylcarbamate had no significant effect on total amounts of extractable epicuticular lipids, nor did it alter the structure of the wax formation on the leaves. The scanning electron microscopy micrographs indicated that *S*-ethyl dipropylthiocarbamate significantly reduced wax formation on pea leaves. 2-Chloroallyl diethyldithiocarbamate altered the structure of the wax formations, but not the total amount of wax (scanning electron microscopy). Trichloroacetic acid had little effect on wax deposition compared to diallate or *S*-ethyl dipropylthiocarbamate (scanning electron microscopy). The implication of the effect of the carbamates on epicuticular lipids and penetration of subsequent topically applied chemicals is discussed.

Surface lipids (or waxes) are the first plant barrier encountered by any material applied to the foliage. For a compound to penetrate into the plant it must spread over the surface of the leaf, stem, or fruit, and then pass through the surface lipid, the cuticle, the cell wall, and into the cytoplasm of the epidermal cells. Stomatal penetration may also be a method of entry into the plant, but so far there has been no satisfactory proof that this is a predominant mechanism of transport into plant tissues. Stomatal penetration is further complicated by the continuous cuticular layer enclosing the substomatal chamber (20). This layer would remain a prime barrier to penetration.

Studies have shown (4, 6, 7, 22) that the contact angle between the solution applied and the plant surface is a method of measuring the wettability of the plant surface by the solution. This contact angle is markedly affected by the degree of roughness or smoothness of the plant surface; the smooth surface is easer to wet and gives greater contact between the solution and the plant surface. The net result should be increased penetration of the compound into the plant.

Epicuticular lipids consist of a variety of substances and take a variety of semicrystalline patterns (3, 11, 12). Electron microscopy has revealed that these patterns appear to be consistent for each plant species, with differences found between species. Attempts are being made to utilize surface lipid components for taxonomic classification (2, 3, 9). Some of the components of surface lipids are hydrocarbons, wax esters (long chain alcohol esters of long chain acids), primary and secondary alcohols, ketones and fatty acids. As a rule, the hydrocarbons are *n*-paraffins (10–50% in plants) containing 21 to 35 carbon atoms in chains with odd number of carbons predominating (3).

Trichloroacetic acid (1, 11, 15) and EPTC[3] (6, 7) have been shown to reduce epicuticular wax. EPTC had no effect on the composition of lipids from sicklepod petioles, although the thickness of the petiolar cuticle was reduced by 35% at the strongest concentration tested (23). Several other pesticides have been screened for their abilities to inhibit or stimulate synthesis of plant lipids (19).

Preliminary investigations in our laboratory showed that two carbamates of different chemical structure exerted completely opposite effects on the susceptibility of pea plants (*Pisum sativum*) to propanil. Peas were treated with CIPC or with diallate. After several days the treated plants were sprayed with varying concentrations of propanil. The treated plants responded to the propanil in contrasting ways; the diallate-treated plants were killed more readily than the controls, and the CIPC-treated plants were less susceptible to propanil than were the controls. The results of the preliminary experiments led us to investigate the chemical and physiological effects of CIPC and diallate in pea plants.

This paper describes experiments designed to determine if these differences in susceptibility could be explained by differences

[1] Use of trade names is for the purpose of identification of equipment employed and does not constitute endorsement by the United States Department of Agriculture.

[2] A portion of these studies is part of the thesis submitted to the Department of Biochemistry, North Dakota State University, in partial fulfillment of the requirements for the Ph.D. Degree.

[3] Abbreviations: EPTC: *S*-ethyl dipropylthiocarbamate; propanil: *N*-(3,4-dichlorophenyl)propionamide; CIPC: *N*-(3-chlorophenyl)isopropylcarbamate; diallate: *S*-(2,3-dichloroallyl)diisopropylthiocarbamate; CDEC: 2-chloroallyl diethyldithiocarbamate; SEM: scanning electron microscope; GLC: gas-liquid chromatography.

in epicuticular lipids. Perhaps the amounts, chemical composition, and structure of the semicrystalline wax formation are altered enough for the propanil to enter diallate-treated pea plants more readily than the controls. By the same means, the CIPC-treated plants may prevent the penetration of propanil because of changes in epicuticular lipids.

MATERIALS AND METHODS

Plant Material. Peas (*Pisum sativum* L., var. Little Marvel) were grown in aerated half-strength Hoagland's nutrient solution, in pint jars or stainless steel trays, in controlled environment chambers or in greenhouses. The chambers were maintained at 27 C to 21 C day to night temperatures, 50% relative humidity, and 1600 ft-c on a 12-hr photoperiod. The greenhouses were kept as close to these conditions as possible; fluorescent light was used to supplement natural light when needed. The plants were treated with the carbamates 12 days after germination and harvested 7 days later. At the time of treatment, leaf 4 (Fig. 1) was still folded and had not begun to expand. At harvest time this leaf on control plants was fully expanded.

Treatment. In some experiments the peas were root-treated, and in some diallate treatments a vapor phase was used. For root treatment the pesticides were dissolved directly in deionized water with gentle heat and constant stirring for 1 to 3 days. Some plants were treated with diallate vapors under transparent Mylar hoods with small exhaust fans venting to the outside. Cotton plugs in 2- × 15-cm glass tubes were inserted through holes near the bottoms of the chambers. A few drops of liquid diallate were added to the cotton. The plants were then exposed to a constant low velocity flow of air containing diallate vapors. Exact dosage rates could not be determined by this method, but the response of the plants was readily monitored by the shiny appearance of the younger leaves within 1 to 3 days. Controls were grown under identical conditions, but without diallate present.

Extraction of Surface Lipids. In initial experiments the waxes were extracted at room temperature with hexane followed by chloroform. The plants were cut between leaves 3 and 4, and waxes were extracted from the two separate parts of the shoot. This was done to permit comparison of the effects of the test compounds on only the wax that was formed after treatment (assuming that the epicuticular wax already formed would not be significantly altered). The tissues were immersed in hexane for 10 sec, the hexane was drained off, and chloroform was added for 10 sec. Anhydrous Na_2SO_4 was added to the extracts to remove water. The solvents were evaporated under vacuum at temperatures below 40 C and the wax was dried to a constant weight and expressed on a dry weight basis. All waxes were stored under nitrogen at 4 C prior to chemical analyses.

In later experiments, $CHCl_3$ was the only solvent used for wax extraction. The shoots above leaf 3 were dipped once for 10 sec and treated as described above. Studies by others (15) have shown that approximately 99% of the wax on leaf surfaces can be extracted by this technique. Also, the reduced time of exposure to the solvents decreases the chance of extracting internal lipids.

Silicic Acid Chromatography. BioSil A, 200 to 325 mesh (BioRad Lab., Richmond, Calif.), washed with hexane, ether, and methanol, activated under reduced pressure at 138 C for a minimum of 18 hr, was poured as a hexane slurry to give a prepared column of 12 × 400 mm. The flow rate of the solvents through the column was 2 ml/min. The hexane solution of the extracted wax was pumped onto the column. The system was rinsed with 20 ml of hexane, and a gradient elution of hexane and ether was pumped through the column. A nine-chamber gradient elution system was used with 50 g of solvent in each chamber. Chambers 1, 2, 3, 4, and 8 contained 100% hexane; chambers 5 and 6 contained 50% (w/w) ether in hexane; chambers 7 and 9 contained 100% ether. After the gradient elution, 100 g of ether followed by 200 g of methanol were pumped through the column. The eluate was collected, and the contents of the tubes were concentrated to 1 to 2 ml. The concentrate was spotted on thin layer chromatography plates to determine the degree of separation and the identity of the compound. The column separated the hydrocarbons, fatty acids, primary alcohols, and unidentified polar constituents at a purity greater than 95%. The esters and secondary alcohols were eluted as a mixture.

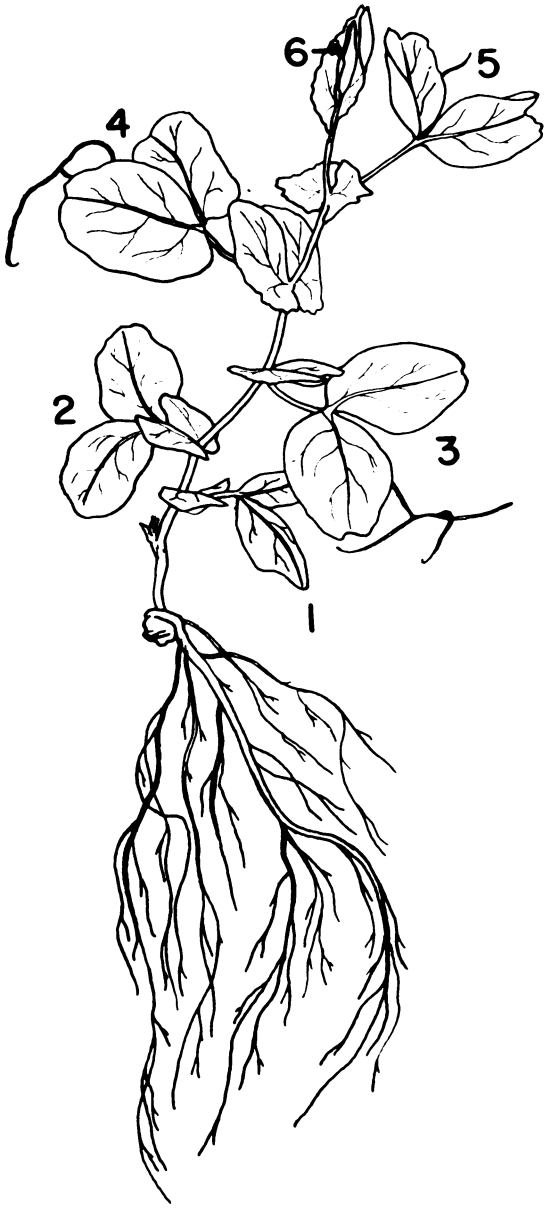

FIG. 1. Diagram of a pea plant at the time of harvest. The numbers indicate the position of each leaf. For studies comparing wax formation in the younger tissues to the older tissues the plants were separated between leaves 3 and 4.

The ester and secondary alcohol mixture was chromatographed on a second column of BioSil A, which was previously washed as described above but not activated. The esters were eluted from the 12- × 150-mm column with 50 to 100 ml of hexane. The secondary alcohol fraction was eluted with ethyl ether. These esters and secondary alcohols were 95% pure as determined by thin layer chromatography. Total mass recovered from these columns was greater than 80%. In all column chromatographic

separations, an equal mass of extracted plant lipids was placed on each column whether the lipids were from control or treated plants.

Thin Layer Chromatography. The concentrated eluates from silicic acid column chromatography were spotted on 20- × 20-cm glass plates, spread with 0.25 mm thick Silica Gel HF and developed with hexane-ethyl ether-acetic acid (70:30:1.5) (v/v). The plates were air-dried, and the spots were made visible by spraying the plates with 5.0% (w/w) $K_2Cr_2O_7$ in 40% (w/w), H_2SO_4 and heated in an oven at 130 C for 20 min. Benzene was used as a developing solvent to separate the hydrocarbons from the esters.

Gas-Liquid Partition Chromatography. A gas chromatograph equipped with a dual ionization flame detector was used for separation of the individual surface lipid components. A 1.8-m × 0.4-cm column containing 3% (w/w) OV-1 on 80/90 mesh gas CHROM Q as inert support was used in all cases cited. The hydrocarbons were separated by temperature programming from 175 to 310 C at 2 C/min, the secondary alcohols from 175 to 320 C at 3 C/min, and the primary alcohols from 220 to 320 C at 4 C/min. The programs were started at the time of sample injection. The chromatograms were quantitated by triangulation. Each peak was tabulated as a percentage of the area of all components present in that lipid fraction.

Mass Spectrometry. The mass spectra were obtained from a Varian M-66 mass spectrometer with a direct inlet system. Samples for mass spectral analysis were collected from the gas chromatograph by using a splitter system with a split ratio of 10:1, in favor of the collector.

Microscopic Observations. Examination of the structure of wax deposited on leaf surfaces of treated and untreated peas was done in two ways: (*a*) a carbon replica technique similar to that described by Juniper (11, 12); and (*b*) scanning electron microscopy. The first method was to evaporate carbon onto the surface of the pea from carbon rods perpendicular to the leaf surface at a vacuum of 10^{-5} to 10^{-4} Torr. The surface was then flooded with 2% cellulose nitrate (Parlodion) in amyl acetate. Copper grids were placed onto the wet surface, and the solvent was evaporated. When dry, the grids were removed from the leaf and inverted onto filter paper, and amyl acetate was added to the filter paper to dissolve away most of the Parlodion, leaving the carbon replica adhering to the grid. The replicas were examined in an RCA EMU-3H electron microscope with no further treatment.

The SEM was carried out at Newport Beach, California. The leaves to be sampled were cut at the petiole as close as possible to the main stem of the plant. Throughout all manipulations considerable care was taken to avoid any contact of the sample area with objects or solvent vapors that might induce changes in the surface features. The leaves were carefully taped to the inside surface of 9-cm plastic Petri dishes. A few drops of distilled water were added, and the dishes were sealed to prevent the leaves from desiccating. The leaf samples were kept cool (but not frozen) with two cans of artificial ice wrapped in the same package. One or 2 days were required for transport of the leaves. The leaves were sampled immediately or stored at 5 C for a maximum of 1 day before observation. Some leaves were dewaxed by four successive 4-sec immersions in $CHCl_3$ just prior to observation in the SEM.

Six-millimeter diameter circles were cut from the lamina of the leaves, sealed to metal holders, and placed into a vacuum chamber. A uniform coating of approximately 500 A of gold-palladium was evaporated onto the surface. The samples were then observed in a Cambridge Mark IIa Stereoscan SEM. Pictures of uncoated leaves from control plants were unsatisfactory. However, they were similar enough to pictures from the metal-coated surfaces that all subsequent studies were made on metal-coated leaves.

RESULTS

Physiological Observations

CIPC Effects. CIPC had no significant effect on the amounts of wax (per gram tissue dry weight) extracted from pea leaf surfaces even though the physiological status of the plants was markedly altered at concentrations of CIPC greater than 10 μM. The same results were obtained whether the entire shoot or only the younger tissues (above leaf 3) were extracted. At 46 μM CIPC, the dry weights of the treated plants were 89% of controls, shoot and root lengths were 63 and 69%, respectively, and the surface areas of individual leaflets (leaf 4) were 66% of controls.

The microscopic observations support the extraction data. Many carbon replicas were obtained from CIPC-treated leaves. The wax structures resembled controls and resembled those published by others (12).

A SEM was used for observation of much larger areas of leaf surfaces, for comparison to replicas, and to obtain a more realistic three-dimensional effect. Figures 2, a and b, show the adaxial and abaxial surfaces, respectively, of leaf 4 from a control plant. Structures were seen that we interpret as wax "crystals." These structures are larger on the abaxial side than on the adaxial side.

The SEM micrographs obtained from leaves of CIPC-treated pea plants were similar to the controls (Fig. 2, a and b) giving further evidence that CIPC does not significantly alter wax deposition on pea leaves. If CIPC induces a change which serves as a protective mechanism against propanil, as we proposed, that mechanism is not due to physical changes in the structure or amount of epicuticular wax.

Diallate Effects. The epicuticular wax was significantly reduced on peas treated with diallate without changing the growth habit of the plants. None of the measured growth parameters were significantly changed at the highest concentrations of diallate used (10 μM). Less than 50% of the extractable wax was recovered from the upper shoots (above leaf 3) with hexane followed by $CHCl_3$ (Table I). When the plants were treated with diallate vapors and extracted only with $CHCl_3$, the wax recovered was only about 18% of the controls (Table II).

The SEM revealed (Fig. 2, e and f) that little or no surface wax crystals were formed on the leaves when peas were treated with diallate. These leaves resemble those in which the wax was removed by $CHCl_3$ (Fig. 2, c and d). The results were the same from plants treated by the roots or with vapors. Our failure to obtain carbon replicas of wax formations on leaves from diallate-treated plants substantiates these results.

Effects of Other Compounds. SEM studies were made of peas treated with trichloroacetic acid and EPTC, two compounds previously reported to alter epicuticular wax formation (1, 6, 7, 11, 13, 15). CDEC was also tested because of its similarity in structure to EPTC and diallate. The plants were root-treated with 10 μM EPTC, 10 μM CDEC, or 100 μM trichloroacetic acid.

The effects of trichloroacetic acid are shown in Figure 3, a and b; of CDEC in Figure 3, c and d; and of EPTC in Figure 3, e and f. Only EPTC treatment gave a significant reduction of wax formation on both leaf surfaces. The physical appearance of the wax formations may have been altered by both trichloroacetic acid and CDEC, but the results so far are inconclusive. It is significant that the most effective of the compounds tested was EPTC, which has a chemical structure most closely resembling diallate.

Qualitative and Quantitative Analyses of the Components of the Epicuticular Lipids from Pea Plants. Qualitative and quantitative analyses of the epicuticular lipids extracted from pea plants showed to a 5- to 6-fold decrease in the surface lipids in those plants which had been treated with diallate vapors (Table II). Table III shows the percentage distribution of these lipid com-

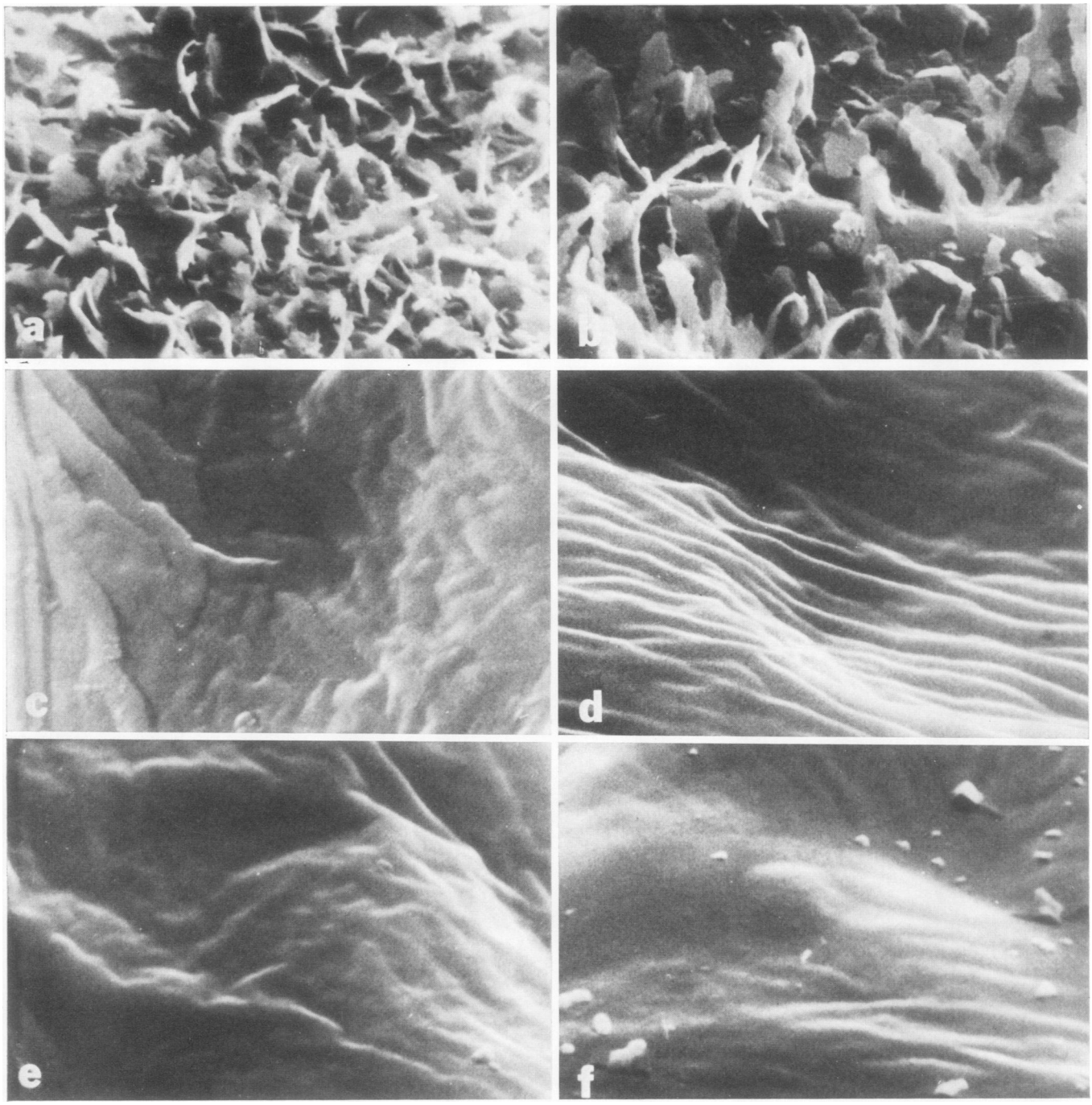

FIG. 2. SEM micrographs of pea leaf surfaces. a: Adaxial surface of leaf 4 from untreated plant. × 10,200. b: Abaxial surface of the same leaf. × 10,450. c: Adaxial surface of pea leaf 4 dewaxed by immersion in chloroform. × 11,000. d: Abaxial surface of the same leaf. × 10,550. e: Adaxial surface of pea leaf 4 treated with diallate vapors. × 9,730. f: Abaxial surface of the same leaf. × 9,320.

ponents from treated and untreated plants. The distribution of the controls is in good agreement with the results of Kolattukudy (17) and Macey and Barber (18). Our ester fraction probably contains some aldehyde. Although the total production of epicuticular lipids was reduced more than 80% with diallate vapors, the percentage distribution of the hydrocarbons, esters, secondary alcohols, and fatty acids was not significantly changed. Diallate treatment significantly decreased the percentage of primary alcohols and increased the percentage of undetermined polar material in the extracted wax.

The hydrocarbon, secondary alcohol, and primary alcohol fractions were analyzed to determine the character of the individual substituents of these groups and whether there were alterations in their ratios as a result of the carbamate treatment. Table IV reports the percentage distribution within each hydrocarbon fraction as a function of carbon number. No significant difference in the long chain *n*-alkane distribution, C_{29} to C_{33}, was observed between controls and diallate-treated plants. In the diallate-treated plants, the C_{31} alkane represents 95% of the C_{29} to C_{33} alkanes, a value in good agreement with the control. The C_{31} component was identified as an *n*-alkane, *n*-hentriacontane, by mass spectrometry from both control and diallate-

Table I. *Effect of Diallate on Epicuticular Wax from Peas*

The plants were grown in nutrient culture in stainless steel trays. Each group of plants was grown in an isolated greenhouse. Treatment with diallate was via the roots. The shoots were separated between leaves 3 and 4. Wax was extracted with hexane for 10 sec followed by $CHCl_3$ for 10 sec.

Concn of Diallate	Lower Shoots		Upper Shoots	
	Hexane	$CHCl_3$	Hexane	$CHCl_3$
μM	*mg wax/g dry wt*			
0	8.7 ± 1.1	3.2 ± 0.3	9.1 ± 1.4	4.6 ± 1.7
0.1	10.1 ± 3.5	3.2 ± 1.6	9.3 ± 2.5	4.2 ± 1.4
1.0	9.7 ± 1.8	4.7 ± 3.7	6.3 ± 0.3	3.0 ± 1.7
5.0	17.7 ± 12.9	4.8 ± 4.0	4.7 ± 0.3	1.6 ± 0.4
10.0	12.8 ± 7.2	0.8 ± 0.9	4.5 ± 1.1	1.3 ± 0.1

Table II. *Effect of Diallate Vapors on $CHCl_3$-extractable Epicuticular Wax from Peas*

These plants were exposed to diallate vapors for 7 days. They were cut between leaves 3 and 4 and immersed in $CHCl_3$ for 10 sec. Only the part of the plant above leaf 3 was extracted.

Treatment	No. of Replications	No. of Plants	Wax
			mg wax/g dry wt
Controls	4	400	16.2 ± 2.8
Diallate	8	800	2.9 ± 0.3

treated plants. The presence of a large molecular ion (21) and the absence of any other intense peak in the higher mass sections of the spectra (3, 13) indicate the absence of branching in the molecule. The contribution of short chain (C_{17} to C_{19}) *n*-alkanes and the large contribution (34.5%) of the polar unidentified materials (Table III) indicates that internal lipids may have been extracted from the diallate-treated tissues which are denuded of their epicticular lipid (10, 14). It is likely that more internal lipids and unidentified polar constituents may be extracted from diallate-treated material as compared to the control. This results from the reduction of the epicuticular lipid boundary in the diallate-treated plants.

Table IV shows the distribution of primary alcohols in surface lipids from pea plants. The chain length of the most abundant primary alcohol was determined by mass spectral analysis and shown to be a C_{26} carbon chain. The C_{26} component was found to be *n*-hexacosanol. Peaks in the high mass range at (M-18), (M-20), and (M-46), were found due to loss of water, loss of water and hydrogen, and loss of water and ethylene, respectively (21). A plot of the GLC retention time *versus* chain length in the C_{24} to C_{30} region gave a straight line that was parallel to the straight line from the standard *n*-alkane chromatogram, indicating that the primary alcohols were straight chain compounds (8), and gave a correlation for the chain length of the other primary alcohols.

Table IV shows the distribution of the secondary alcohols isolated from the pea leaf surface lipids. The chain length of the most abundant component (C_{31}) was obtained from the mass spectrum. The same graphical method of chain length determination was used for the secondary alcohols as described for the primary alcohols. The structure of the C_{31} component was found by mass spectrometry to be a mixture of isomeric secondary alcohols. No molecular ion peak was observed, but a peak at (M-2), a large peak at (M-18), and a small peak at (M-46) were recorded. These peaks are all characteristic of alcohols. The appearance of peaks at (M-183), (M-197), (M-211), (M-225), and (M-239) due to the ion $R-CH{=}OH^+$ (where R− is $C_{17}H_{35}-$, $C_{16}H_{33}-$, $C_{15}H_{31}-$, $C_{14}H_{29}-$, and $C_{13}H_{27}-$, respectively) indicate that the alcohol is secondary and a mixture of isomers (21). These five peaks suggest the presence of the three secondary alcohols, hentriacontan-16-ol, hentriacontan-15-ol, and hentriacontan-14-ol, with approximate abundances of 55, 39, and 5%, respectively, based on the relative intensities of the above-mentioned peaks. These values are in agreement with values published by Macey and Barber (18).

DISCUSSION

Previous investigations (7, 19) showed that both thiocarbamate and dithiocarbamate compounds were capable of altering the quantity of epicuticular lipids on plant leaves. Dewey *et al.* (1), Juniper (11), and Kolattukudy (15) demonstrated the ability of trichloroacetic acid to alter the amount of epicuticular waxes on plants. Several of these investigators described the importance of the epicuticular wax layer for maintenance of water balance by plants, and Gentner (7) described the increased toxicity of a contact herbicide to cabbage after treatment with the thiocarbamate EPTC. A similar response was observed by us when pea plants were treated with another thiocarbamate, diallate. These plants became increasingly sensitive to the herbicidal action of foliarly applied propanil with increased diallate concentration. An opposite response to propanil was observed when pea plants were pretreated with CIPC. In many cases, plants pretreated with CIPC were found to be less sensitive to propanil than were the control plants. Little evidence is presented here to account for the effects of CIPC, but this paper presents extensive evidence to support the hypothesis that the thiocarbamate diallate inhibits the biosynthesis of pea epicuticular lipids.

Plants grown in the presence of diallate were not significantly changed in outward appearance with the exception that the leaves became shiny. This shiny appearance was primarily the result of decreased epicuticular wax. This was substantiated by the fact that the extractable epicuticular lipids from vapor-treated plants was only one-fifth the quantity obtained from controls (Table II), and when the plants were root-treated, the younger tissues yielded less than one-half that obtained from controls (Table I). The decreased inhibition of wax formation in root-treated plants as compared to vapor-treated plants may be due to less diallate reaching the sites of lipid synthesis.

Electron microscope studies support the quantitative data. The failure to produce carbon replicas of diallate-treated tissues suggests that there is no structure on the surface of these leaves. The SEM micrographs of diallate-treated pea show few crystalline structures on the epicuticular surfaces of the treated plants. The similarity of the SEM micrographs from treated tissues and dewaxed control tissues gave further evidence that the diallate-treated tissues were denuded of much of their epicuticular lipids. The results of the SEM do not rule out the possible presence of some epicuticular wax on the leaf surface. It may be that some wax covers the surface as a continuous sheet, but with no "crystalline" structure.

The SEM confirms the previously reported observation that EPTC does inhibit epicuticular lipid biosynthesis (6, 7). No quantitative comparison between EPTC and diallate is possible from our data. However, it is clear that both thiocarbamates inhibit wax formation in pea plants. The dithiocarbamate, CDEC, appears to have little effect upon the production of epicuticular lipids at the concentration used in our experiments. The significance of the alteration of the crystalline structure by CDEC is unknown. From our data no comparison can be made between the dithiocarbamate, CDEC, and the thiocarbamates, diallate and EPTC, but we can speculate a structure-activity

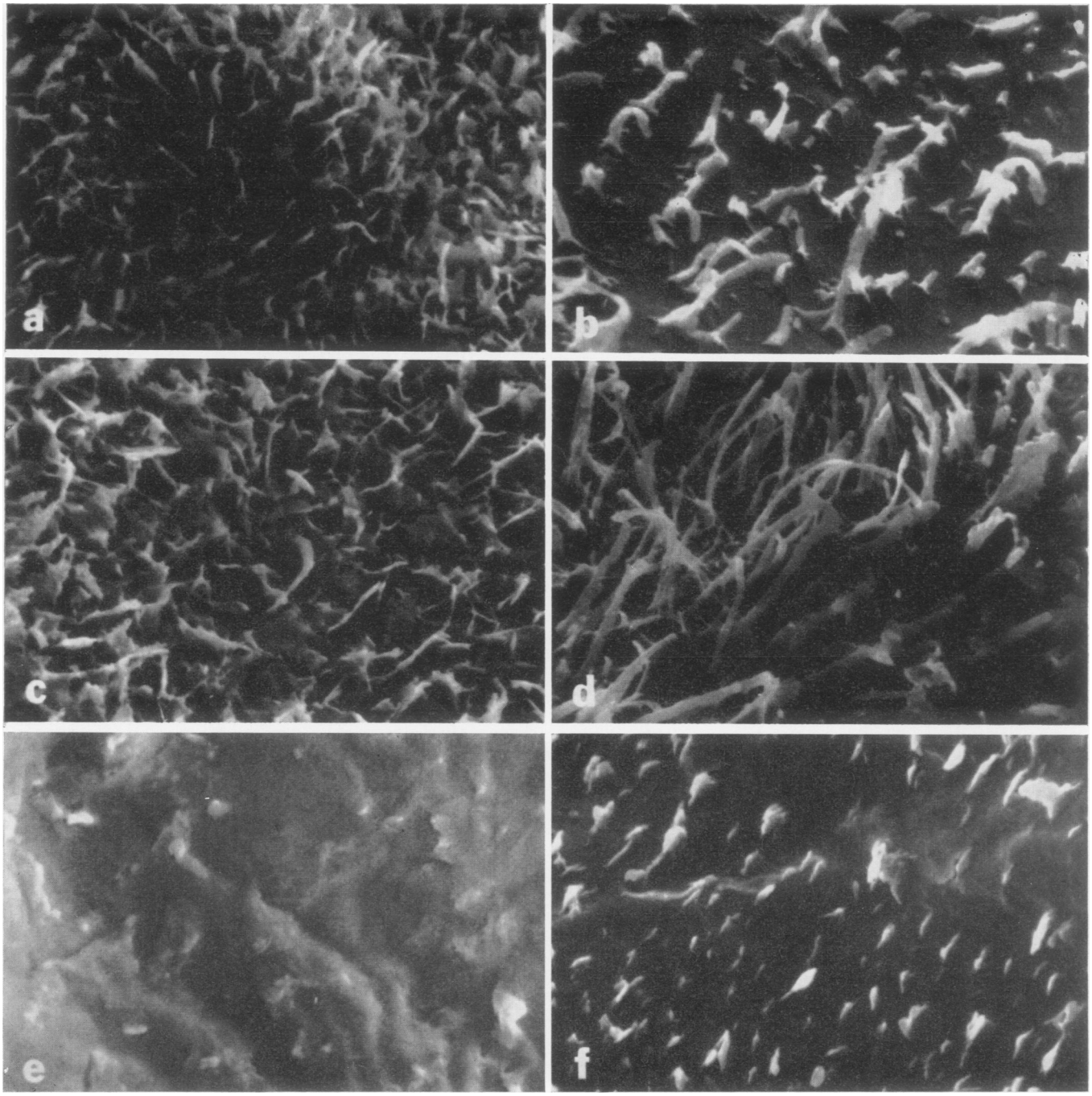

FIG. 3. SEM micrographs of pea leaf surfaces from plants root-treated with trichloroacetic acid, CDEC or EPTC. a: Adaxial surface of leaf 4 treated with 100 μM trichloroacetic acid. × 9,800. b: abaxial surface of the same leaf. × 9,970. c: Adaxial surface of leaf 4 treated with 10 μM CDEC. × 9,800. d: Abaxial surface of the same leaf. × 9,440. e: Adaxial surface of leaf 4 treated with 10 μM EPTC. × 9,900. f: Abaxial surface of the same leaf. × 9,550.

relationship between these molecules. EPTC and CDEC have an *n*-alkane substitution at the nitrogen of the carbamate while diallate has an isoalkyl substitution. Diallate and CDEC are chlorinated allyl thioalcohols while EPTC is a primary thioalcohol. CDEC does not inhibit surface lipid biosynthesis, but EPTC and diallate are both inhibitory. Thus, the substitution of the nitrogen or the thioalcohol does not seem to be responsible for inhibition, as the different substituents appear in both the active and inactive molecules. Therefore, the carbonyl group may be the functional portion of diallate and EPTC. The substitution of sulfur for oxygen in the thiocarbonyl of CDEC may render this molecule inactive. Investigation is underway to determine what effect carbamate structure has on the biosynthesis of epicuticular lipids.

The decreased susceptibility of pea plants pretreated with CIPC cannot be explained by significant changes in crystalline wax formation nor by increases of extractable epicuticular lipids. Significant changes in plant growth were found in CIPC-treated plants at concentrations greater than 10 μM. The decreased susceptibility of CIPC-treated plants to propanil may be due to

Table III. *Distribution of the Components of Epicuticular Lipids from Pea Plants Extracted 10 sec in Chloroform*

	Control	Diallate Vapor
	%	%
Hydrocarbons	47.8	40.3
Esters	7.0	8.9
Secondary alcohols	5.8	5.4
Fatty acids	2.2	5.6
Primary alcohols	26.5	5.3
Unidentified[1]	10.9	34.5

[1] All compounds with an R_F lower than the primary alcohols, as determined by thin layer chromatography with the solvent system hexane-ether-acetic acid (70:30:1.5) (v/v).

Table IV. *Distribution of Three Components in the Epicuticular Lipids of Pea Plants Extracted 10 sec in Chloroform*

Analysis was by use of gas-liquid chromatography on a 1.8-m × 0.6-cm, 3% OV-1 column. The temperature program used was 175 to 310 C at 2 C/min for the *n*-alkanes, 175 to 320 C at 3 C/min for the secondary alcohols and 220 to 320 C at 4 C/min for the primary alcohols. The carbon chain length of each component of the *n*-alkanes was obtained by interpolation of its retention time from a plot of standard *n*-alkanes, C_{18} to C_{36} *versus* retention times. The method of chain length determination for the secondary alcohols and primary alcohols is given in the text.

No. of Carbon Atoms in Chain	*n*-Alkanes		Secondary Alcohols		Primary Alcohols	
	Control	Diallate	Control	Diallate	Control	Diallate
	%	%	%	%	%	%
17	...	0.5	...	...	...	...
18	...	0.8	...	...	...	...
19	...	0.6	...	...	...	...
23	...	...	0.9	3.8	...	...
24	...	...	...	...	1.3	...
25	0.3	1.6	...	...	...	...
26	...	...	0.6	1.2	63.2	40.9
27	...	0.8	...	...	...	...
28	...	...	...	...	34.5	44.8
29	0.9	1.5	0.6	1.5	...	...
30	0.7	0.9	0.5	0.7	1.0	12.9
31	95.8	90.2	95.4	90.5	...	...
32	0.8	0.8	0.4	0.2	...	...
33	1.7	1.6	0.6	0.5	...	...

decreased leaf size, resulting in a smaller target area for the foliar application. However, other preliminary experiments with simultaneous root treatment of CIPC and propanil indicate that this is not the case. CIPC may induce chemical alterations within the plant that result in decreased susceptibility to propanil. Pea plants may contain an enzyme responsible for detoxification of propanil. Inhibition of this enzyme by CIPC in peas could be similar to the action of *N*-methyl carbamates and organophosphates on the aryl acylamidase in rice (5).

Diallate vapors inhibited the production of primary alcohols to a greater extent than they inhibited the other surface lipid components (hydrocarbons, esters, secondary alcohols, and fatty acids). Even though the treated plants showed an 80% reduction in epicuticular lipids, the ratios between the *n*-alkanes, esters, secondary alcohols, and fatty acids were unchanged.

The predominant paraffin *n*-hentriacontane (C_{31}), the predominant secondary alcohol, hentriacontanol (C_{31}), and the presence of the homologous series in both the *n*-alkane and the secondary alcohols are in good agreement with the findings of others (17, 18). The ratio of hydrocarbon to secondary alcohols within the treated plants was similar to the ratio found in the controls (Table III). This indicates that the block in the biosynthesis of the epicuticular wax must occur prior to formation of the precursor of the *n*-hydrocarbons and secondary alcohols. If diallate affected the elongation-decarboxylation mechanism, the predominant hydrocarbon should have been an *n*-alkane of shorter chain length than C_{31}. This was not the case. Furthermore, this block would result in a similar alteration of the homologous series observed in the secondary alcohol fraction.

Diallate appeared to alter the production of the long chain primary alcohols (Tables III and IV). Diallate-treated plants contained only one-fifth of the primary alcohol found in control plants, and the C_{26} to C_{28} ratio was altered in the treated tissues. Wollrab (24) and Kolattukudy (15) postulated that the *n*-alkanes and ketones are produced in the epidermis but that the fatty acids are synthesized at a different site. Further, Kolattukudy has demonstrated a precursor-product relationship between the *n*-alkane and the secondary alcohols (P. E. Kolattukudy, 1970, manuscript in preparation). Our data show that the primary alcohols are inhibited by diallate treatment to a greater extent than are other waxy lipid components. This suggests that the block of epicuticular lipid biosynthesis is at a site separated from the elongation-decarboxylation mechanism for the biosynthesis of the *n*-alkane and secondary alcohols. It is not clear why the fatty acids are affected differently from the alcohols, but further investigation is in progress to elucidate the mechanism by which diallate inhibits the synthesis of the primary alcohols and the epicuticular lipids of pea plants.

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