Inhibition of Epicuticular Wax Deposition on Cabbage by Ethofumesate

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ABSTRACT

The weight of epicuticular wax on the surface of cabbage (Brassica oleracea var. Capitata 'Market Prize') leaves was reduced by soil treatments of ethofumesate (2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate) and EPTC (S-ethyl dipropylthiocarbamate). Separation of epicuticular wax into major components by gas-liquid chromatography indicated that ethofumesate decreased the deposition of n-nonocosane and n-nonocosa-15-one on cabbage leaves but increased the deposition of a minor component, the long chain waxy esters. EPTC was less inhibitory to n-nonocosane-15-one deposition than was ethofumesate. EPTC did not increase long chain waxy ester deposition. Scanning electron micrographs revealed that ethofumesate almost totally eliminated the epicuticular wax on cabbage leaves while EPTC only diminished it. Cuticular transpiration was increased by ethofumesate but not by EPTC. Ethofumesate appears to be a more potent inhibitor of epicuticular wax deposition than EPTC.

Dewey et al. (1) first reported in 1956 that a herbicide inhibited epicuticular wax deposition. They showed that trichloroacetic acid inhibited epicuticular wax on peas (Pisum sativum L.) and Brassica crops. The trichloroacetic-acid-treated plants had glossy foliage that showed increased susceptibility to foliar applied herbicides and an increased rate of transpiration. Transmission electron micrographs of pea leaf surfaces showed that trichloroacetic acid reduced the number and form of minute wax structures on leaf surfaces (4).

In 1966 Gentner (3) reported that the herbicide EPTC also decreased epicuticular wax deposition, increased spray retention, and increased transpiration rates of cabbage (Brassica oleracea var. Capitata L.). Flore (2) reported the EPTC decreased the total amount of epicuticular wax on cabbage leaves, preferentially decreasing the amount of long chain alkanes and ketones while increasing the amount of long chain waxy esters.

Purdy and Turner (8–10) reported that n-nonocosane made up 93% of the alkanes they isolated from the surface wax of cabbage and n-nonocosen-15-one made up 70% of the ketones. The alkanes and ketones were the two most abundant compounds in cabbage wax, with less long chain waxy esters and sec-alcohols also present. This has been confirmed since by Laseter et al. (7) using combined gas chromatography-mass spectrometry. The inhibition of leaf wax deposition by trichloroacetic acid, EPTC, and other herbicides has been used as a tool by Kolattukudy (5, 6) in the establishment of the elongation-decarboxylation mechanism of epicuticular wax synthesis.

The purpose of this study was to examine the effects of a new experimental herbicide, ethofumesate, on epicuticular wax deposition on cabbage. The study was initiated following the observation that ethofumesate-treated sugar beet (Beta vulgaris L.) emerged with glossy leaves.

MATERIALS AND METHODS

Plant Culture and Herbicide Application. Cabbage (B. oleracea var. Capitata 'Market Prize') plants were grown in soil that received preplant incorporation treatments with ethofumesate at 0.84 and 2.24 kg/ha and EPTC at 0.84 and 3.36 kg/ha. EPTC was included in the experiments as a reference since it is a known inhibitor of epicuticular wax deposition on cabbage. Both herbicides were sprayed on the surface of 880 ml of soil (greenhouse mix 1:1:2:1 soil, peat, fine sand, Vermiculite) in a 946-ml waxed food cup. The herbicides were incorporated into the top 2.5 cm of soil immediately after application and three cabbage seeds planted in each cup 0.14 cm deep in the soil. The cabbage plants were grown in the greenhouse supplemented with artificial lighting to provide a 12-hr day/12-hr night. Greenhouse temperatures ranged from a minimum of 20 C at night to a maximum of 33 C during the day.

Gravimetric Determination of Epicuticular Wax. When the cabbage plants were in the sixth leaf stage, the fourth and fifth leaves were removed, placed in the bottom of a 1-liter beaker, and washed twice for 10 sec with 100 ml of glass-distilled chloroform. The area of the leaves was measured with an automatic area meter (Lambda Instruments). A 75-ml aliquot of the chloroform-epicuticular wax extract was filtered through Whatman No. 1 filter paper into preweighed 80-ml aluminum tart pans (10-cm diameter). After the solvent was evaporated for 18 hr at room temperature, the pans were reweighed. The weight of epicuticular wax per cm² leaf area was calculated. Values reported are the means of two experiments, four replications/treatment, with 12 leaves harvested/repllication.

Gas-Liquid Chromatography (GLC) of Epicuticular Wax. The effects of ethofumesate on wax quality as measured by the relative deposition of n-nonocosane, n-nonocosa-15-one, and long chain waxy esters (36–44 carbons total) on cabbage leaf surfaces were determined by GLC. According to Purdy and Turner (8) and Flore (2) these compose 65 to 70% of the chloroform-soluble extract. EPTC was again included as a reference. Values are the mean of four replications. The GLC system used was a Beckman GC-4 with a hydrogen flame detector interfaced with a recorder and a Digital PDP 11 computer. The column was 1.7 m in length and packed with 3% Dexsil 300. The column temperature was 320 C, the detector temperature 360 C, and the inlet line 260 C. N₂ was used as the carrier gas. Elution times of the selected wax
components were determined from a previously identified cabbage wax sample (2). Separation into major chemical classes was achieved by TLC using 250-µm Silica Gel G plates prewashed in benzene, dried at 100°C, then loaded with sample and developed in benzene.

The separation was visualized by spraying a 2.5-cm strip with 34% H2SO4 acid and heating to 160°C for 15 min. Strips not subjected to visualization adjacent to visualized spots were scraped, eluted with 5 ml of chloroform, dried, and redissolved in 1 ml of chloroform for GLC analysis.

Samples were obtained from the previously described gravimetric wax determinations. After the solvent from a 25-ml aliquot of the chloroform-wax solution was evaporated at room temperature under a forced air stream, the wax was redissolved in 1 ml of chloroform for GLC. The areas of the peaks formed by n-nonacosane and n-nonacosan-15-one were measured by computer, converted to a per cm² leaf area basis, and expressed as per cent of control. The heights of the peaks formed by the long chain waxy esters were manually measured and summed. This sum was converted to a cm² leaf area basis and expressed as a per cent of control.

Scanning Electron Microscopy (SEM). Scanning electron micrographs were made with an International Scientific Instruments Super Mini-I SEM of the adaxial surface of fresh cabbage leaves. Micrographs were made of the third leaf when plants were in the fourth leaf stage. Only leaves from the control and highest rates of herbicide treatments were used. Rectangular leaf pieces (4 × 8 mm) were cut from each leaf at one side of the midvein near the leaf center. The leaf pieces were placed, abaxial surface down, upon the adhesive side of a strip (1 × 1 cm) of silver metallic tape (Scotch slide masking tape) that had previously been glued (with Tube-Koat) to the top of an aluminum SEM stub. The stubs were then placed in the SEM and micrographs taken on Polaroid type 107 film at 5 kv acceleration potential. The entire process took less than 15 min from the time the leaf pieces were first cut.

Cuticular Transpiration. The effect of ethofumesate on the rate of transpiration through the cuticle was determined by measuring the rate of water loss from excised cabbage leaves. When cabbage plants were in the eight-leaf stage, leaves six and seven were excised and placed in 10-cm diameter aluminum tart pans. A completely dewaxed control was prepared by immersing leaves from untreated plants into a 100-ml bath of chloroform for 15 sec. The pan plus leaves was weighed immediately and then placed in an exhaust hood. The pans were reweighed after 1, 1.5, 2.75, 6, and 26.5 hr in the hood. The weight of water lost from the leaves during each time period was calculated. A regression coefficient (equal to the per cent water loss/hr) was calculated for each treatment from the water loss data from time zero until 90% of the original water was lost or 26.5 hr, whichever came first. Stomata on the excised leaves closed after 0.25 hr. Values are the mean of three replications. The experiment was repeated with similar results.

### RESULTS AND DISCUSSION

Preplant-incorporated ethofumesate treatments significantly decreased the amount of epicuticular wax on the surface of cabbage leaves similar to EPTC (Table I). There were no significant differences observed between the effects of the high and low rates of either herbicide (Table I). SEM of cabbage epicuticular wax from both treated and nontreated cabbage leaf appear as an interconnected network of wax projections (Fig. 1, A and B). This network was almost entirely eliminated by ethofumesate and greatly diminished by EPTC (Fig. 1, C and D). Although differing considerably in structure, ethofumesate had a similar effect on epicuticular wax deposition as reported for EPTC. Because of this similarity, ethofumesate appears to be a promising tool in the investigation of lipid metabolism. Further research is needed to determine whether synthesis of internal cytoplasmic lipids is also inhibited by this compound.

The major components of the cabbage leaf wax were separated by GLC into peak 1 with a retention time of 0.61 min identified as a C-29 alkane, peak 2 with a retention time of 0.89 min identified as a C-29 ketone, and a group of these peaks with retention times over 6 min which were C-36+ esters. Ethofumesate at both rates significantly decreased the deposition of n-nonacosane and n-nonacosan-15-one on cabbage leaves as compared to the control (Table II). Ethofumesate also significantly increased the deposition of long chain waxy esters, a minor component, over that of the control. EPTC significantly decreased the deposition of n-nonacosane and n-nonacosan-15-one but did not significantly increase that deposition of long chain waxy esters. Ethofumesate at 0.84 kg/ha inhibited the n-nonacosan-15-one more than did the same rate of EPTC. EPTC induced increase in long chain waxy esters and decrease in n-nonacosane and n-nonacosan-15-one as evidence that EPTC inhibits chain elongation of fatty acids as proposed by the elongation-decarboxylation mechanism for wax synthesis proposed by Kolattukudy (5, 6). The increase in long chain waxy esters may be caused by the accumulation of short chain (C-16 to C-22) fatty acids and primary alcohols when the elongation-decarboxylation mechanism is blocked by EPTC. Ethofumesate, because it increased the deposition of long chain esters and decreased n-nonacosane and n-nonacosan-15-one, could therefore also inhibit chain elongation of fatty acids in the elongation-decarboxylation pathway of epicuticular wax synthesis.

The gravimetric determination of epicuticular wax demonstrated no differences in the weight of epicuticular wax deposition between cabbage treated with ethofumesate and EPTC. However, ethofumesate not only decreased n-nonacosan-15-one more than EPTC, it also increased the long chain waxy esters, whereas EPTC did not.

The cuticular transpiration of cabbage leaves was increased 7.8 times by treatment with 2.27 kg/ha ethofumesate. The chloroform-washed leaves transpired at 40 times the rate of the nonwashed

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<tr>
<th>Table I. Influence of herbicide treatment on epicuticular leaf wax deposition on cabbage.</th>
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<tbody>
<tr>
<td>Treatment</td>
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<tr>
<td>Control</td>
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<td>EPTC</td>
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<tr>
<td>Ethofumesate</td>
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<td>Ethofumesate</td>
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1 Values with the same letter or letters are not significantly different at the 5% level using the Least Significant Difference Test with prior significant F-test. Downloaded from on October 1, 2017 - Published by www.plantphysiol.org Copyright © 1978 American Society of Plant Biologists. All rights reserved.
control. EPTC did not significantly affect the rate of cuticular transpiration. Ethofumesate also decreased the amount of wax observed on fresh cabbage leaves to a greater degree than did EPTC. Thus, ethofumesate appears to be a more potent inhibitor of epicuticular wax deposition than EPTC.

Acknowledgment  We wish to thank J. Flore, Michigan State University, for his technical advice and for samples of n-nonocosane and n-nonocosan-15-one.

LITERATURE CITED

1. Dewey OR, J Gregory, RK Pfeiffer 1956 Factors affecting the susceptibility of peas to selective dinitro herbicides. Proc 3rd Br Weed Conf 1: 313-326

![Fig. 1. SEM of adaxial surface of fresh cabbage leaves. A: cabbage leaf (× 1,000); B: cabbage leaf (× 5,000); C: cabbage leaf treated with 3.36 kg/ha EPTC (× 1,000); D: cabbage leaf treated with 2.24 kg/ha ethofumesate (× 1,000).](image)

Table II. Effect of ethofumesate and EPTC on major cabbage wax components

| Herbicide     | Rate (kg/ha) | % of control
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<tbody>
<tr>
<td></td>
<td>n-nonocosane</td>
<td>n-nonocosan-15-one</td>
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<tr>
<td>Control</td>
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<td>100.0 c</td>
</tr>
<tr>
<td>EPTC</td>
<td>0.84 21.8 b</td>
<td>37.4 b</td>
</tr>
<tr>
<td>Ethofumesate</td>
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<td>18.2 a</td>
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<tr>
<td>Ethofumesate</td>
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<td>12.3 a</td>
</tr>
<tr>
<td>Ethofumesate</td>
<td>2.24 2.4 a</td>
<td>7.2 a</td>
</tr>
</tbody>
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1 Values within columns with the same letter or letters are not significantly different at the 5% level, using the Least Significant Difference test with prior significant F-test. For the control, n-nonocosane composed 45%, n-nonocosan-15-one composed 50%, and long chain waxy esters composed 1% of the material recovered by the GLC system.