Absorption of *S,S,S*-Tributylphosphorotrithioate by Cotton Leaves

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The combined techniques of microscopic time-lapse photography and autoradiography were used in a series of experiments which indicated that *S,S,S*-tributylphosphorotrithioate would directly enter readily through the cuticle of the upper epidermal cells of cotton leaves. Partial plasmolysis, cuticular vesication, vesicle collapse, and progressive blackening of palisade cells accompanied the absorption. Blistered epidermal cells and blackened palisade cells were fewer in the leaves of a resistant selection than in the leaves of a Deltapine (susceptible) variety after treatment with individual droplets.

S,S,S-tributylphosphorotrithioate, $(BuS)_3PO$, is a cotton defoliant which is applied principally as an aqueous emulsion. At ambient temperatures, it is a relatively nonvolatile, oily liquid that has a distinct odor. Katterman and Hall (1961) reported that its application to cotton leaves was followed by an increase in respiration and reduction in free sulfhydryl content. They credited $(BuS)_3PO$ as being the first thiophosphorus compound reported to induce and accelerate abscission. They further stated that $(BuS)_3PO$ is apparently degraded directly to water-soluble compounds.

The absorption of numerous compounds has received considerable attention in recent years. Several excellent reviews are available (Epstein and Jefferies, 1964; Franke, 1967; Van Overbeek, 1956; Woodford *et al.*, 1958); however, relatively little information is reported pertaining to defoliants and even then, the evidence is indirect. Brun *et al.* (1961) concluded that tributyl phosphate and other defoliating chemicals entered the leaves of several woody species through the stomates. Their conclusions were based on the fact that desiccation occurred more rapidly when the compounds were placed on the lower stomatous surfaces of the leaves than when placed on the upper astomatous surfaces.

In our studies, the principal objective was the determination of the mode of entry of $(BuS)_3PO$ into cotton leaves. With the use of microscopic time-lapse photography augmented by autoradiography, an increased understanding of surface distributions and cellular responses was obtained. Further evidence obtained with the time-lapse series suggested that under our experimental conditions the principal mode of entry was directly into the upper epidermal cells.

MATERIALS AND METHODS

Gossypium hirsutum L. (variety Deltapine 15 and selection G-272) plants were grown in peat moss and Perlite potting mixture with added dolomitic limestone. The plants were watered with a solution containing 2.5 grams of 15–30–15 fertilizer per liter, with the addition of microelements as necessary. The G-272 selection was included because of its resistance to $(BuS)_3PO$-induced defoliation (Miller and Endrizzi, 1962). $(BuS)_3PO$ will usually induce defoliation of Deltapine 15 plants under favorable temperature conditions. All applications were made with 1.9% (v./v.) aqueous emulsions of the commercial product or with the sulfur-35-labeled material formulated with the same organic, xylene-type solvents and the same emulsifiers as the commercial material (trade name DEF, Chemagro Corp., Kansas City, Mo.). The oldest, mainstem leaves on mature plants at least 3 months old were used for the sequences.

Microscopic Time-Lapse Photography. A Bolex, H-16 Rex-2 16-mm. movie camera, controlled by a Zieler Instruments time-lapse drive and timers, was fitted to a microscope for use in the single droplet penetration sequences. Capillary tubes were used to place individual 1- to 2-mm. diameter droplets of the emulsion on the upper epidermis of the leaves of both types of plants. The leaves, still attached to the plants, were held in place

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on the microscope substage by a special spring clamp. Ambient temperatures remained at 25° C., with the relative humidity below 50%. Supplemental incandescent light was adjusted so that the leaf under observation received between 400 and 600 foot-candles of constant illumination. After placement of the droplet, the time-lapse sequences were initiated at the rate of one frame per minute with the use of intermittent $^1/_2$- to $^3/_4$-second exposures of light.

The usual procedure was to transport the plants from the greenhouse in the early afternoon for treatment. No attempts were made to determine if the stomates were open at time of treatment. The treated area of the leaf was illuminated with incident light for the first 24 hours, then with transmitted light for the remainder of the time to record changes in the palisade cells and vascular tissue.

The incident-lighted sequences were projected on a screen for measurement of droplet areas, diameters of epidermal cells, and accumulated number of blisters at different time intervals for calculation of the numbers per square millimeter and per cent of total cells. The transmitted-lighted sequences were projected for measurement of the diameters of palisade cells and recording of final number of blackened palisade cells. The use of a stage micrometer for the microscope enabled translation of screen-projected sizes to square millimeters. Only the areas under the original droplets were considered in the calculations.

Gross Autoradiography. Ten leaves per plant on each of two plants of both cotton strains were treated in the greenhouse with six 0.004-ml. droplets of an aqueous emulsion of $(BuS^{35})_3PO$ (measured specific activity 0.444 μc. per ml.) on the upper epidermis of each leaf. Individual leaves were collected from both types of plant at intervals of 2, 4, and 6 hours and 1, 4, and 8 days after treatment. The harvested leaves were exposed to No-screen x-ray film for 2 months at −20° C.

Leaf Disk Autoradiography. Six leaves per plant on each of three plants of both strains were treated in the greenhouse with six 0.001-ml. droplets of an aqueous emulsion of $(BuS^{35})_3PO$ (specific activity 0.515 μc. per ml.) on the upper epidermis of each leaf. India ink was used to mark the location of the droplets. Three duplicate sets of 1.5-cm. diameter disks were removed from the leaves 10 and 30 minutes, 1 and 4 hours, and 1, 4, and 8 days after application of the droplets. At each interval of time, one set of disks was rinsed by dipping into three separate beakers of distilled water, while another set remained unwashed. The fresh disks were mounted on 5 × 5 cm. glass plates and exposed to medium contrast 5 × 5 cm. lantern-slide plates for one month at −20° C. All of the plates were developed in the same holder prior to examination and densitometric measurement of the autoradiographs were made with a Spinco Model R Analytrol fitted with a film densitometer attachment. The plates were superimposed on their respective leaf disks and observed microscopically.

RESULTS

Microscopic examination of the droplets indicated that the $(BuS)_3PO$ globules coalesced as the aqueous phase of the droplets disappeared. The $(BuS)_3PO$ was irregularly deposited on the surface of the leaf. Greater quantities tended to be deposited over the concave surfaces of secondary veinlets, around glandular hairs, at the base of true leaf hairs, around dust particles, and in depressed areas between epidermal cells.

Microscopic Time-Lapse Photography. Microscopic time-lapse photography indicated that individual interstomatal epidermal cells lost turgor (Figure 1) at various intervals after application of $(BuS)_3PO$. Approximately one minute after completion of the turgor loss, vesication occurred, which resulted in a raised area over the epidermal cell. The appearance of multicellular vesicated areas (Figure 2) and loss of distinction of epidermal cell junctures (Figure 1) indicated that the vesication was cuticular.

There was no statistically significant difference in the times of initial vesicle formation between the G-272 and the Deltapine 15 leaves. In both types, the vesication

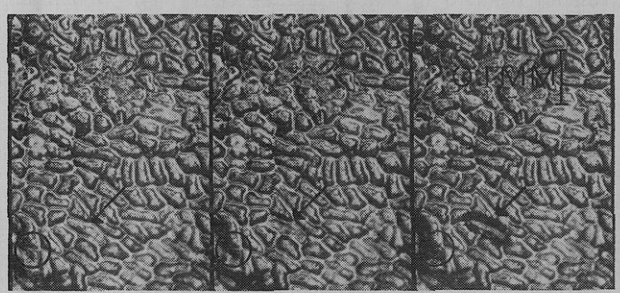

Figure 1. Upper surface of Deltapine cotton leaf

Photomicrographs taken at 1-minute intervals show sequence of turgor loss and vesication resulting 20 minutes after application of a single droplet of $(BuS)_3PO$

Left. Essentially normal convex appearance of cell, designated by arrow
Center. Same cell after turgor loss
Right. Same cell after vesication
○ Loss of distinction of depressed area over anticlinal wall between epidermal cells

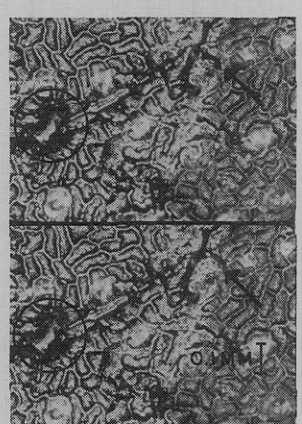

Figure 2. Upper epidermis of G-272 cotton leaf

Photomicrographs taken at 1-minute intervals show multicellular vesicated area in circle over veinlet. Vesicle collapse shown by arrows. Left-hand two thirds of view is area under treatment with single droplet of $(BuS)_3PO$

continued for an average of 8 hours or more (Figure 5); however, many of the vesicles subsided as new ones were formed. The collapse of the vesicles, in many cases, occurred within a minute (Figure 2), even though the vesicle may have been raised for 4 or more hours. Individual epidermal cells apparently were damaged prior to vesication. Further information was obtained through viewing the films which cannot be expressed graphically—namely, that the formation of blisters did not proceed from a stomate and extend radially. The blisters rarely occurred in the epidermal cells adjacent to the stomates, except where blister formation progressed in a front or wave across the viewing area and resulted in blistering of essentially all of the epidermal cells. The degree of stomatal opening could not be determined through projection of the films.

Generally, shortly after the cuticular vesicle subsided, there was a progressive blackening of the palisade cells located directly beneath the vesicle; however, not all vesicles resulted in black palisade cells. The maximum number of black palisade cells observed after application of a droplet to a Deltapine leaf was 293 per sq. mm. or approximately 5% of the total number of palisade cells under the droplet. There was no subsequent blackening of palisade cells adjacent to an individual malanoid cell within 2 to 3 days' observation of either type of plant. Blackening occurred only under previously cuticularly vesicated epidermal cells.

Vascular discoloration was observed in isolated areas of some veinlets. It did not increase laterally in the vascular tissue but changed progressively from pale yellow to brown.

Gross Autoradiography. The placement of individual droplets of $(BuS^{35})_3PO$ on leaves of Deltapine and G-272 plants indicated that neither $(BuS^{35})_3PO$- nor sulfur-35-containing metabolic products were translocated to any appreciable extent within 8 days after application.

Leaf Disk Autoradiography. Observation of the semimicroautoradiographs superimposed on their corresponding leaf disks indicated that there was no specificity of the $(BuS)_3PO$ for guard cells or substomatal cavities. The irregular deposit and lack of specificity are illustrated in Figure 3. Densitometric measurements of the autoradiographs (Figure 4) indicated that the maximum rate of absorption of S^{35} coincided with the maximum rate of blister formation (Figure 5). The autoradiographic measurements served as indirect evidence that the cause of the blisters was the $(BuS)_3PO$ and not the solvent carriers, but did not provide absolute proof.

DISCUSSION

Two factors indicated that $(BuS)_3PO$ readily entered directly into the epidermal cells. Both were derived from direct observations. First, individual interstomatal vesicated epidermal cells occurred with no apparent injury to the cells between the individual vesicated cell and surrounding stomates and vesication did not proceed radially from cells adjacent to the guard cells. Second, the semimicroautoradiographs showed no apparent specificity of the $(BuS)_3PO$ for guard cells or substomatal cavities. For illustration of substomatal cavity accumulations the work of Dybing and Currier (1961) serves as an excellent example.

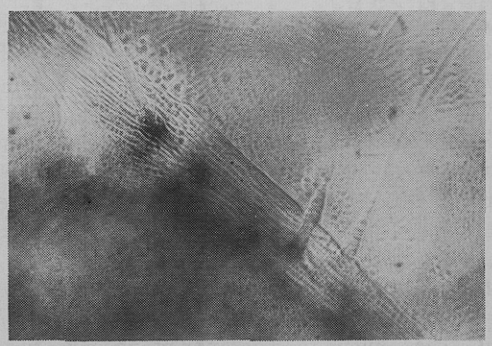

Figure 3. Photomicrograph of autoradiograph of leaf disk

Deposit of $(BuS^{35})_3PO$ 10 minutes after application and washing of a G-272 selection leaf. Leaf hairs and other details are result of impressions formed in emulsion of lantern slide plate

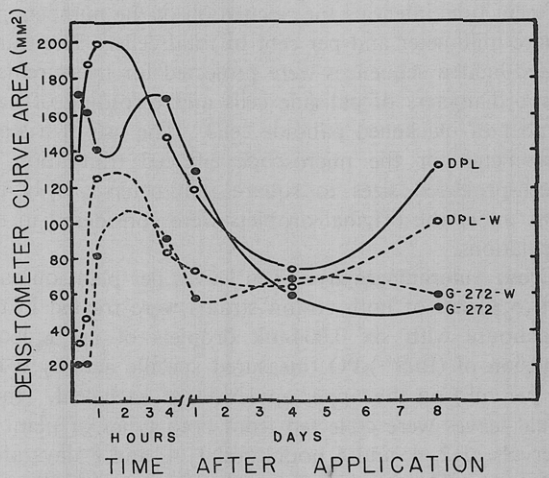

Figure 4. Areas under densitometrically measured curves from semimicroautoradiographs of leaf disks from leaves treated with single droplets of $(BuS^{35})_3PO$

DPL. Deltapine, unwashed
DPL-W. Deltapine, washed
G-272. G-272 selection, unwashed
G-272-W. G-272 selection, washed

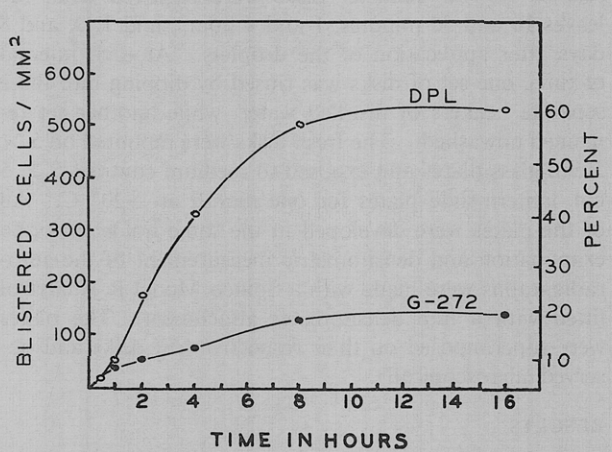

Figure 5. Accumulated total number of blistered epidermal cells from Deltapine and G-272 selection cotton leaves derived from evaluation of microscopic time-lapse films

The densitometric curves indicate that the initial rate of retention of the S^{35} was greater in the Deltapine than in the G-272 leaves; however, after a few hours there was no difference in the amounts retained. There was, however, a difference in the response between the two types from the standpoint of amount of blisters formed and number of blackened palisade cells. The most logical explanation is that either there was a differential fixation of the $(BuS)_3PO$ in the cuticle or a detoxification mechanism existed to a greater extent in the G-272. In either event, the evidence suggests that as the $(BuS)_3PO$ entered the epidermal cell, it produced partial plasmolysis, followed shortly by cuticular vesication. The $(BuS)_3PO$ apparently diffused into the palisade cells about the time that the cuticular vesicle subsided.

The subsequent lack of diffusion of S^{35} from the site of placement of the original droplet as indicated by macro- and semimicroautoradiography, coupled with the lack of progression of blackening from one palisade cell to surrounding ones, suggests that the action of the $(BuS)_3PO$ in inducing abscission is indirect. Possibly during the injury, some substance or substances are produced by the tissue which are translocated to the abscission zone to induce abscission.

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