Technical Papers

Toxicity of Cellulose Acetate Sheets to Plants and Fish

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In a series of experiments on insect transmission of plant viruses carried out in 1949, cellulose acetate cages were used for confining the insects on plants. Transparent cellulose acetate sheets, 0.020 in. thick, obtained from a commercial house, were used. When crimson clover plants (*Trifolium incarnatum* L.) were placed under the cages they developed characteristic lesions on the leaflets within 3 days (Fig. 1, *a, b, c, d*), and most of them died within 2 weeks.

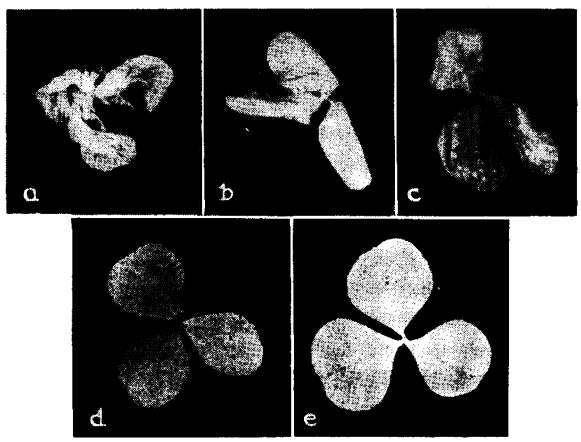

FIG. 1. *a, b, c, d*: Leaflets of crimson clover showing lesions and burning symptoms caused by toxic cellulose acetate sheets; *e*: normal leaflet. (Photographs by A. J. Carlile.)

Sheets of cellulose acetate from other sources were tested, and the material from one proved to be nontoxic. A spectrophotometric analysis[1] of different kinds of cellulose showed that the toxic sheets contained diethyl phthalate.

On inquiry it was learned that most manufacturers of cellulose acetate sheets used diethyl phthalate as the plasticizer. A comparison of the effects of toxic cellulose acetate cages and c. p. diethyl phthalate was therefore made. Crimson clover plants were caged under bell jars with small vials containing diethyl phthalate. Lesions similar to those produced by the toxic cages appeared on the leaflets. When a single leaflet was brought in contact with diethyl phthalate, the plant developed characteristic lesions and died within 3–5 days. The toxic effect was also tested on aster (*Callistephus chinensis* Nees) and tobacco (*Nicotiana tabacum* L.). Aster plants were found less susceptible, whereas tobacco proved to be more sensitive.

[1] The author is indebted to George I. Lavin and Herbert Jaffe for the spectrophotometric analyses.

Storage, or soaking the sheets in water, had little or no effect on the toxicity of cellulose acetate. Since toxic cellulose acetate tasted bitter it could easily be distinguished from the nontoxic material. Diethyl phthalate is bitter, has a boiling point of 295° C, and is nearly insoluble in water. It was found that soaking toxic cellulose acetate cages in alcohol, in which diethyl phthalate is miscible, removed most of the toxicity but caused so much shrinking of the material that it became unsuitable for use in the construction of insect cages.

The toxic effects of diethyl phthalate and of cellulose acetate sheets containing it are not limited to plants. Fish were exposed to the cellulose acetate material known to be toxic to plants, by confining them in water in a fishbowl in which small pieces of the material were immersed. They died within a few hours, despite the low solubility of diethyl phthalate in water. For a more precise test, sheets were cut into pieces, approximately 300 mg each, and 1–50 pieces were immersed in Erlenmeyer flasks containing 50 ml water. Three g of the material killed a 1½-in. goldfish within 45 min, 0.3 g within 4½ hr, and 1 drop of diethyl phthalate in 50 ml water within 30 min. Cellulose acetate powder and sheets of nontoxic cellulose acetate, as well as cellulose nitrate sheets, had no toxic effects on plants or fish. The results indicate that small amounts of diethyl phthalate are highly toxic to both fish and plants.

In a limited number of tests with mice, adding toxic cellulose acetate sheets to their drinking water produced no harmful effect.

Manuscript received November 6, 1951.

The Distribution of S^{35}-Labeled L-Methionine Sulfoximine in the Rat[1]

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It has been shown by various investigators (*1–3*) that methionine sulfoximine is the toxic agent produced by treating prolamines with nitrogen trichloride. The sulfoximine has been isolated from agenized flour and recently synthesized (*4, 5*).

When injected or fed to susceptible animals, convulsions may develop after a period of several hours, and the animals may die if the dose is high enough. There is evidence that methionine sulfoximine is a methionine antagonist, and Reiner (*6*) has shown that the convulsions produced by the drug in rabbits may be delayed or suppressed by feeding excess methionine.

[1] This project has been supported by the Office of Naval Research, Navy Department.

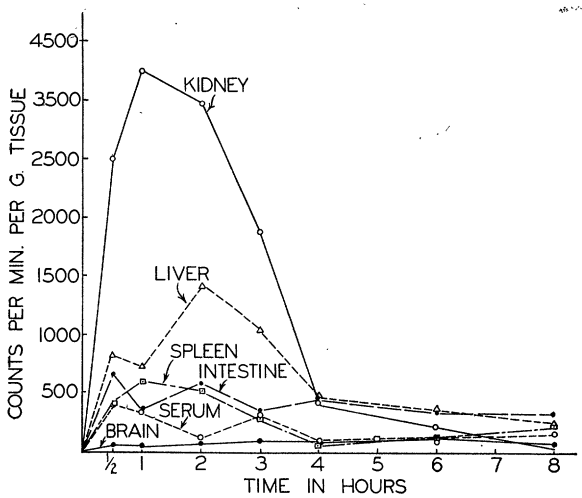

FIG. 1. Distribution of S^{35} L-methionine sulfoximine in rat tissues.

Inhibition of the growth of *Leuconostoc mesenteroides* (*7*) is also reversed by extra methionine. If the nervous symptoms are indeed caused directly or indirectly by a methionine antagonist, this would suggest that methionine has some as yet unknown function in nerve metabolism. If the distribution of methionine sulfoximine in the animals affected by the drug could be determined, this might give a clue to its mode of action. The work described below was undertaken in order to obtain information bearing on these questions.

In the synthesis of S^{35}-labeled L-methionine sulfoximine, several modifications of the original method (*4*) have been made; S^{35}-labeled DL-methionine (84 mg = 1.5 mc) was diluted with 10 g of inactive DL-methionine, the mixture benzoylated, and then resolved enzymatically by the method of Dekker and Fruton (*8*). The first crop of crystals of S^{35} L-methionine weighed 1.04 g and had a specific activity of 21,000 cpm/mg as counted in a gas flow Geiger tube. The L-methionine (1.04 g) was converted to the sulfoxide by the method of Toennies and Kolb (*9*). The yield was 0.96 g. A chromatogram was run, using 0.05% butanol-acetic acid, and this gave R_f ascending 0.13. The R_f of pure methionine sulfoxide is 0.13. Two faint additional spots were nonradioactive. The S^{35} methionine sulfoxide (0.96 g, 0.007moles) was placed in a 15-ml three-necked flask equipped with dropping funnel, stirrer, and gas delivery tube, the end of the tube being placed several millimeters below the surface of water in a beaker. The flask was cooled in an ice bath, and 2 ml of concentrated H_2SO_4 was added with stirring. When most of the sulfoxide had dissolved, the temperature was raised to 45° C by means of a water bath, and 0.60 g (0.014 moles) of HN_3 dissolved in chloroform was added over a period of 1 hr, the temperature being maintained between 45°–50° C. (The HN_3 concentration should be 1.5 *N* or greater.) The progress of the reaction may be followed by the evolution of nitrogen. When all the HN_3 had been added, the stirring was continued for several hours, and the mixture allowed to stand overnight. A drop of the reaction mixture was adjusted to pH 5.5, and chromatograms were run as before and compared to pure methionine sulfoximine. No methionine sulfoxide remained. (If the chromatogram indicates the presence of sulfoxide, additional HN_3 may be added.) The reaction mixture was poured into a small amount of cracked ice and brought to pH 5.5 with $BaCO_3$. The $BaSO_4$ formed was filtered and washed with hot water until the washings gave a negative ninhydrin test. The combined filtrate and washings were then evaporated *in vacuo* to about 5 ml. Any precipitate is filtered, and the filtrate was further evaporated nearly to dryness. The residue was dissolved in the least possible quantity of hot water, and an equal volume of methyl alcohol added. On cooling in the refrigerator overnight crystals of methionine sulfoximine formed. They were recrystallized from a small quantity of hot water. The yield was 0.434 g of the sulfoximine having the same specific activity as the L-methionine.

Uptake of S^{35} L-methionine sulfoximine by rat tissues. Eight rats were injected intraperitoneally with 14–15 mg of the sulfoximine and then autopsied at intervals up to 8 hr. After 2–3 hr the rats not sacrificed developed convulsions, or incipient convulsions, and one died at 8 hr. Tissues investigated were liver, kidney, spleen, intestine, brain, spinal cord, and serum. Samples of each were digested with Pirie's reagent (*10*). The sulfate formed was precipitated as barium sulfate and then assayed according to the method of Boursnell *et al.* (*11*), using, however, a gas flow Geiger tube with standard geometry. The activities of the tissues are shown in Fig. 1. Activity was highest in the kidney, but this probably represents a very high excretion of the drug in the urine. Several urine samples were collected after 4–5 hr, and they showed activities up to 40% of the injected dose. At the end of 8 hr kidney activity had fallen almost to background, but liver and intestine still contained moderate activity, suggesting actual incorporation of the radioactive sulfoximine, or a derivative of it, in these tissues. The low uptake by brain and spinal cord

TABLE 1

UPTAKE OF S^{35} DL-METHIONINE BY LIVER SLICES WITH AND WITHOUT ADDED DL-METHIONINE SULFOXIMINE

With methionine sulfoximine			Without methionine sulfoximine		
Sample No.	Cpm/g liver slice	μg S^{35} DL-methionine taken up/g tissue	Sample No.	Cpm/g liver slice	μg S^{35} DL-methionine taken up/g tissue
1	14,570	51	6	32,200	133
2	10,380	36	7	33,700	139
3	9,340	33	8	31,100	129
4	18,170	63	9	33,900	140
5	15,410	54	10	22,760	94
Av	13,574 ± 1640	48 ± 6		30,732 ± 1050	127 ± 9

during the period of the experiment is of considerable interest.

To test the effect of DL-methionine sulfoximine on the uptake of S^{35}-labeled DL-methionine by liver slices, they were washed in cold Krebs' isotonic medium for 30 min, blotted dry, and weighed. Approximately 0.5-g samples were incubated in 15 ml Krebs' isotonic solution at pH 7.4 for 130 min at 37° C in an air atmosphere. The media contained (1) 30 mg S^{35} DL-methionine/100 ml, (2) 30 mg S^{35} DL-methionine, plus 30 mg of unlabeled DL-methionine sulfoximine/100 ml. Activity of the methionine was 264,000 cpm/mg as counted in a "Q" gas tube. The results are given in Table 1.

The low uptake of methionine sulfoximine by brain and spinal cord indicates the probable absence of a direct effect of the drug on these tissues. One possible explanation for its action suggested by the results in Table 1 could be through its inhibition of methionine uptake by nerve tissue and thus inhibition of the synthesis of enzymes important to nerve metabolism. Additional work is under way to test this and other possibilities.

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Manuscript received October 5, 1951.

Growth-Regulator Specificity in Relation to Ovary Wall Development in the Fig

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Calimyrna figs produced parthenocarpically by growth-regulator application are seedless (*1–3*). In addition to the fact that embryo development does not take place, the endocarp, or inner ovary wall, of the fruitlets (achenes) does not sclerify and impart hardness to the so-called "seed" as in the case of pollinated syconia (*4*). Parthenocarpic Calimyrna fig syconia produced during the 1951 season with benzothiazol-2-oxyacetic acid,[1] however, have been found to contain achenes with the endocarp tissue as completely sclerified as in syconia resulting from cross-pollination. Thus, parthenocarpic figs produced with this compound have a texture identical to those produced by the usual commercial procedure of caprification (cross-pollination).

These results demonstrate the high degree of specificity of the growth-regulating substances and the diverse reactions they induce. During the past five years, several growth-regulators have been found to induce parthenocarpy in the Calimyrna fig, a variety that requires the stimuli of pollination and fertilization in order for the syconia to set and mature. Ovary wall development of achenes in syconia induced to develop parthenocarpically by three of these compounds has been investigated histologically. In each case some pronounced modification from the normal development and composition of the ovary wall has been revealed. In syconia produced with γ-(indole-3)-*n*-butyric acid, the endocarp in the individual achenes was completely absent (*4*). The endocarp of achenes in syconia produced with *p*-chlorophenoxyacetic acid, on the other hand, was observed to have developed, but typical sclerification of this tissue did not take place. Hence, the tissue did not become hard, but remained parenchymatous in character. Figs produced with this compound have become known commercially to the trade as "miracle seedless figs," because they do not contain achenes which characterize pollinated figs.

Benzothiazol-2-oxyacetic acid, when applied at a concentration of 100 ppm, induced 100% of the unpollinated but pollen-receptive syconia to set and mature parthenocarpically, with no injury to fruits or foliage. Of these, 80% matured at the same time as pollinated syconia, and 20% of the induced parthenocarpic figs reached maturity 3 weeks following the date of spray application, a response similar to that obtained with 2,4,5-trichlorophenoxyacetic acid (*5*). The syconia that matured approximately 6 weeks previous to the time of maturity of pollinated syconia contained achenes, the endocarp tissue of which did not sclerify. The process of endocarp sclerification did not take place, apparently because the time interval between growth-regulator application and maturity of the syconia was too short. Benzothiazol-2-oxyacetic acid-induced parthenocarpic syconia that matured at the same time as pollinated syconia, however, contained achenes with a completely sclerified endocarp. With the exception of slight differences in color of the pulp and lack of both endosperm and embryo development in the achenes, the parthenocarpic syconia appeared identical to pollinated syconia as regards shape, size, and texture.

It is significant that the most pronounced influence of the growth-regulating compounds inducing parthenocarpy in the Calimyrna variety of fig is morphologically manifest in a discrete tissue of the ovary wall. Hence, depending upon the growth-regulator used to induce parthenocarpy, distinctly different achenes can be produced. The following three distinct modifications from normally pollinated syconia, for example, are now possible in the Calimyrna fig: (*a*) syconia containing achenes in which the endocarp is lacking, [produced with γ-(indole-3)-*n*-butyric acid]; (*b*) syconia containing achenes in which the

[1] Supplied by the American Cyanamid Company.