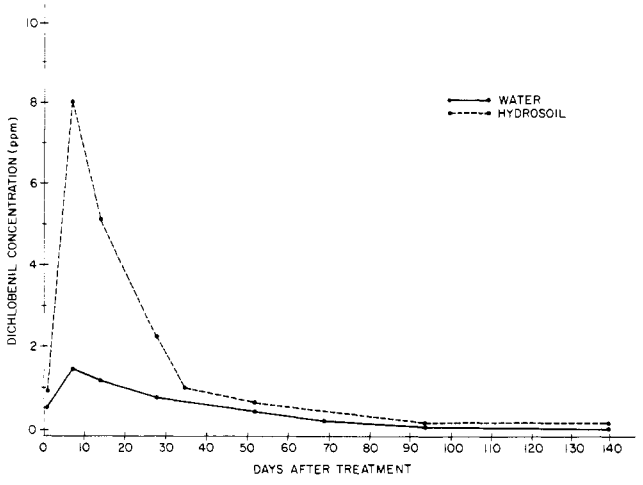

Figure 1. Dichlobenil residues in water and the top 1 in. of hydrosoil of a treated farm pond, with time.

RESULTS AND DISCUSSION

The retention time for authentic dichlobenil was 1.02 min and the lower limit of detection for the herbicide was 0.15 ng. The recovery of dichlobenil from fortified samples of water and hydrosoil averaged 98 and 90%, respectively.

Figure 1 shows the concentration of dichlobenil in the pond water and top 1-in. layer of hydrosoil at various times after treatment. The values appearing in this figure are the average of duplicate determinations. Among the water samples taken, the highest concentration of dichlobenil (1.41 ppm) was detected 7 days after treatment. Thereafter, the concentration of dichlobenil declined gradually, reaching 50% of the maximum detected level 28 days after treatment. Only traces of the herbicide (0.002 ppm) were detected in the water 140 days after treatment. As in the water, the highest concentration of dichlobenil in the hydrosoil (8 ppm) was found in the sample taken 7 days after treatment. Thereafter, a rapid decrease in the concentration of dichlobenil occurred in the hydrosoil and only 22% of the maximum detected level remained 35 days after treatment. This initial, rapid rate of disappearance was followed by a more gradual decrease which paralleled the rate of loss of dichlobenil in the water. The concentration of the herbicide in the hydrosoil had decreased to 0.13 ppm 140 days after treatment when the last sample was taken.

Thus, both in water and hydrosoil, we observed a pattern of increase of the herbicide residues followed by a decrease. The fact that a higher concentration of dichlobenil was detected on the seventh day than on the first day after treatment indicates that the herbicide was not released into the water and hydrosoil immediately after application. Presumably, the lag in reaching the maximum concentration was caused by the slow dissolution of the granular formulation. The disappearance of dichlobenil from the water may be attributed to volatilization (Massini, 1961), microbial degradation (Verloop and Nimmo, 1970), and photodecomposition (Plimmer and Hummer, 1968), whereas microbial degradation, absorption of the herbicide by aquatic vegetation, and possibly leaching to lower depths may have been the contributing factors in the disappearance of dichlobenil from the hydrosoil. The maximum concentration of the herbicide measured in the hydrosoil exceeded that in the water through the first 4 weeks. This may have been due to the tendency of dichlobenil to adsorb to soil (Massini, 1961) and to the low solubility of the herbicide in water.

The results show that small amounts of dichlobenil remained in water and hydrosoil for an extended period under the experimental conditions. The degree of dissipation of the herbicide in our experiment is comparable to that reported for ponds in Oregon and Colorado (Van Valin, 1966; Frank and Comes, 1967; Cope *et al.*, 1969; Ogg, 1972) but is slower than that reported for ponds in Florida (Walsh *et al.*, 1971).

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LITERATURE CITED

Cope, O. B., McCraren, J. P., Eller, L., *Weed Sci.* **17,** 158 (1969).
Frank, P. A., Comes, R. D., *Weeds* **15,** 210 (1967).
Frank, P. A., Hodgson, R. H., Comes, R. D., *Weeds* **11,** 124 (1963).
Massini, P., *Weed Res.* **1,** 142 (1961).
Meulemans, K. J., Upton, E. T., *J. Ass. Offic. Agr. Chem.* **49,** 976 (1966).
Ogg, A. G., Jr., *Pestic. Monit. J.* **5,** 356 (1972).
Plimmer, J. R., Hummer, B. E., Abstracts, 155th National Meeting of the American Chemical Society, San Francisco, Calif., 1968.
Van Valin, C. C., *Advan. Chem. Ser.* **No. 60,** 271 (1966).
Verloop, A., Nimmo, W. B., *Weed Res.* **10,** 65 (1970).
Walker, C. R., *Weeds* **12,** 267 (1964).
Walsh, G. E., Miller, C. W., Heitmuller, P. T., *Bull. Environ. Contam. Toxicol.* **6,** 279 (1971).

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An Analysis of the Phytosterols of Two Varieties of *Cannabis*

The quantitative determination of the 3-β-hydroxysterols in American-grown (MS-13) and Thailand-grown *Cannabis* has been accomplished. The Thailand sample showed no free sterols although it contained the highest total sterol content. No campesterol was found as the glycoside in the MS-13 and Thailand sample while the Thailand sample contained no stigmasterol as the glycoside.

The 3-β-hydroxysterols campesterol, stigmasterol, and β-sitosterol have recently been identified in extracts of *Cannabis* (Doorenbos *et al.*, 1971; Fenselau and Hermann, 1972). These phytosterols have also been found in tobacco (Stedman, 1968; Keller *et al.*, 1969) and have been implicated as precursors of carcinogenic hydrocarbons in its smoke (Wynder *et al.*, 1959). As we have found the phytosterols to constitute approximately 1% of the neutral compounds in the smoke condensate of *Cannabis*, it was of interest to determine their levels in the plant material.

Quantitative analyses of sterol content were performed on *Cannabis* grown at the University of Mississippi (MS-

13 Mexican male) and in Thailand (confiscated material). Levels of glycosidated, esterified, and free sterols were determined gravimetrically by a modification of methods developed for the analysis of sterols in tobacco (Keller *et al.*, 1969; Stedman and Rusaniwskyi, 1959). Duplicate 5.00-g samples of moisture-free *Cannabis*, which had been ground to pass a 60-mesh screen, were extracted with 250 ml of acetone for 24 hr in a Soxhlet apparatus. The extracts were taken to dryness and the residues were treated in one of three ways. (1) Twenty-five milliliters of 95% ethanol containing 0.15 ml of sulfuric acid were added and the solution was refluxed for 15 hr. Fifteen milliliters of 10% KOH in 95% ethanol were then added and the mixture refluxed for 30 min. This treatment yielded total sterols since both glycosides and esters were hydrolyzed. (2) The residue was taken up in 25 ml of 10% KOH in 95% ethanol and the mixture refluxed for 30 min. This treatment gave a value for only free and esterified sterols as glycosides were not hydrolyzed. (3) The residue was taken up in 25 ml of 95% ethanol and refluxed for 5 min. This procedure provided only the free sterols as neither glycosides nor esters were hydrolyzed. Thus, values for free sterols were determined directly and values for glycosides and esters could be calculated by the appropriate subtractions.

The sterols were extracted from the hydrolyzed and unhydrolyzed solutions by adding 50 ml of water and extracting with four 50-ml portions of *n*-hexane. The hexane extracts were combined and washed with three 50-ml portions of 90% methanol, which were in turn combined and back-extracted with two 50-ml portions of *n*-hexane. The hexane fractions were then combined and evaporated to dryness, the residue dissolved in 20 ml of boiling absolute ethanol, and 5 ml of hot 2% digitonin in 80% ethanol was added. After 1 min, 5 ml of water was added to the boiling solution and the mixture was allowed to cool at room temperature overnight. The precipitate was collected on a tared sintered-glass Buchner funnel and washed with several small portions of 80% ethanol followed by diethyl ether. The funnel was dried at 100° for 1 hr and weighed and weights were reproducible to ±8%. The sterol weight was equal to 25.3% of the weight of digitonide precipitate (Wall and Kelley, 1947). As shown in Table I, no free sterols were found in the Thailand sample although it contained the highest total sterol level.

The values for individual sterols were determined by glc (Keller *et al.*, 1969; Grunwald, 1970). The sterol digitonides obtained by the above analysis were decomposed by pouring three 10-ml portions of hot Me_2SO through the funnel (Issidores *et al.*, 1962). After cooling, the Me_2SO layer was extracted with three 50-ml portions of *n*-hexane which were combined and evaporated. The residue was taken up in 1 ml of tetrahydrofuran and samples were injected into a gas chromatograph (Beckman GC-45) using a flame ionization detector. The sterols were separated in a 10 ft × 4 mm glass column packed with 5% OV-101 on GC-Q, 80–100 mesh with a column temperature of 275° and helium carrier gas flow of 75 cm^3/min at 34 psi. Samples were injected on-column and quantitative analysis was performed by electronically integrating the peaks. Reproducibility experimentally determined was to be better than ±6%. Corrections were made for differences in relative weight response as determined from synthetic mixtures. Only campesterol, stigmasterol, and β-sitosterol were found in the sterol fractions from MS-13 plant material although an unknown component which eluted after β-sitosterol was present in small amounts in the Thailand sample. The calculated levels of individual sterols are presented in Table II and it should be noted that no campesterol and stigmasterol glycosides were found in the Thailand sample and no campesterol glycoside in MS-13.

Table I. Free, Esterified, and Glycosidated Sterols in *Cannabis*

	mg of sterol/g of *Cannabis*			
	Glycosides	Esters	Free	Total
MS-13	0.52	0.97	0.37	1.86
Thailand	0.69	1.45	Nil	2.14

Table II. Individual Sterols in *Cannabis*

	mg of sterol/g of *Cannabis*		
	Campesterol	Stigmasterol	β-Sitosterol
MS-13			
Glycosides	0.00	0.01	0.51
Esters	0.12	0.09	0.76
Free	0.06	0.04	0.27
Total	0.18	0.14	1.54
Thailand			
Glycosides	0.00	0.00	0.69
Esters	0.12	0.13	1.20
Free	0	0	0
Total	0.12	0.13	1.89

LITERATURE CITED

Doorenbos, N. J., Fetterman, P. S., Quimby, M. W., Turner, C. E., *Ann. N. Y. Acad. Sci.* **191**, 3 (1971).
Fenselau, C., Hermann, G., *J. Forensic Sci.* **17**, 309 (1972).
Grunwald, C., *Anal. Biochem.* **34**, 16 (1970).
Issidores, C. H., Kitagawa, I., Mosettig, E., *J. Org. Chem.* **27**, 4693 (1962).
Keller, C. J., Bush, L. P., Greenwald, C., *J. Agr. Food Chem.* **17**, 331 (1969).
Stedman, R. L., *Chem. Rev.* **68**, 153 (1968).
Stedman, R. L., Rusaniwskyi, W., *Tob. Sci.* **3**, 44 (1959).
Wall, M. E., Kelley, E. G., *Anal. Chem.* **19**, 677 (1947).
Wynder, E. L., Weight, G. F., Lam, J., *Cancer* **12**, 1073 (1959).

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