Rapid Determination of 4(2,4-DB) and a Metabolite, 2,4-D, in Treated Forage by Electron Affinity Spectroscopy

WALTER H. GUTENMANN and DONALD J. LISK

Department of Entomology, New York State College of Agriculture, Cornell University, Ithaca, N. Y.

A rapid method is described for the determination of 4(2,4-DB) and a metabolite, 2,4-D in treated birdsfoot trefoil–timothy grass forage. The method involves the direct esterification of an acetone extract of the crop with BF_3-methanol. The methyl esters of the herbicides are extracted into hexane and injected into a gas chromatographic column equipped with an electron affinity detector. A sample may be extracted and both herbicides determined in about 45 minutes. The recovery of the herbicides is excellent. The method will detect about 0.2 p.p.m. of 4(2,4-DB) and 0.1 p.p.m. of 2,4-D. The disappearance of both herbicides in the forage is illustrated.

THE HERBICIDE 4(2,4-dichlorophenoxy)butyric acid [4(2,4-DB)] may be used for broadleaf weed control in forage crops. A method for its determination has been developed based on gas chromatography and electron affinity determination of the methyl ester of 4(2,4-DB). The method was used to study the disappearance of 4(2,4-DB) in field-treated, birdsfoot trefoil–timothy grass forage. A peak with the exact retention time of 2,4-dichlorophenoxyacetic acid (2,4-D) appeared [presumably by beta-oxidation of 4(2,4-DB) in the plant] in early samplings and could also be quantitatively determined by the same method. Its formation and disappearance were also followed and are reported here.

Equipment

The gas chromatograph used was a Barber-Colman Model 10 with a battery-operated (*2, 3*) No. A-4071, 6-cc. detector containing 56 μc. of radium-226. The detector was operated at 11 volts which was found to be its optimum for electron capture by chlorinated compounds. A 90,000-megohm resistor was added to the electrometer to give additional gains of 3000; 10,000; and 30,000. The 3000 setting was used exclusively in this study. A 0–50 mv. Wheelco Recorder equipped with 10-inch chart paper running 10 inches per hour was used.

The column was borosilicate glass, U-shaped, 9 mm. o.d. and 6 feet long. The packing was 5% ethyl acetate fractionated Dow Corning high vacuum silicone grease on 80–100 mesh, acid-washed Chromosorb W. Connections between the column and detector were made with metal hypodermic tubing, glass elbows, and silicone rubber through septums. The operating temperatures for the column, flash heater, and detector were 200°, 265°, and 235° C., respectively, and nitrogen (60 cc. per min.) was the carrier gas. The column was conditioned for 16 hours at 230° C. before use.

Procedure

On June, 21, 1962, two 6-foot swaths of birdsfoot trefoil–timothy grass (first cutting), 100 feet long, were treated, respectively, with 1.5 and 3 pounds per acre of 4(2,4-DB), diethylamine salt (Amchem's Butyrac 118) in 30 gallons of water. The application was made with a Willys Jeep equipped with a "Yellow Devil" sprayer with an 18-foot boom consisting of three 6-foot sections. Only the middle section was used in the application.

A large sample of untreated forage was taken at the start of the experiment to serve as check material. Samples totaling about 800 grams were taken daily for 25 days from 15 stations in the treated plots. Later samplings were made at less frequent intervals. The total sample was chopped in a Hobart Food Cutter, thoroughly mixed, and stored in a polyethylene bag at 0° F. prior to analysis.

The method of analysis for 4(2,4-DB) and 2,4-D in forage was as follows:

Twenty-five grams of the crop was weighed in a 250-ml. beaker, and the sample was transferred to a semimicro Waring Blendor jar. One milliliter of 85% orthophosphoric acid was pipetted onto the forage. The beaker was rinsed with 80 ml. of acetone, and the rinsings were transferred to the Blendor. The sample was blended for 2 minutes. The contents were transferred to a sintered glass funnel (40 mm., coarse porosity disk) upon which a thin layer of glass wool had been placed. The sample was filtered by suction into a 125-ml. flask marked at approximately 100 ml. The filter was rinsed with two 20-ml. portions of acetone each time compressing the sample with the bottom of a 50-ml. beaker to squeeze out remaining acetone. The filtrate was poured into a graduated cylinder and the volume adjusted to 100 ml. by evaporation with an air stream or addition of acetone.

One milliliter of the acetone solution was transferred to a 10-ml. volumetric flask and the acetone evaporated using an air stream. Some water from the plant material may still be present but does not interfere. Three milliliters of BF_3-methanol reagent (*5*) was added to the flask, and the flask was held in a boiling water bath for 2 minutes with frequent swirling. The flask was cooled, and 1 ml. of hexane was added. The solution was made to volume with 2% sodium sulfate solution and the flask shaken vigorously for 30 seconds. Up to 10 μl. of the upper hexane layer was injected into the chromatographic column. The retention times for 2,4-D and 4(2,4-DB) were approximately 5 and 10 minutes, respectively (Figure 1A).

The standard curves for the herbicides were developed as follows: zero, 0.2, 0.4, 0.6, and 0.8 ml. of 4(2,4-DB) (1 μg. per ml.) in acetone was pipetted into a series of 10-ml. volumetric flasks. For 2,4-D, the same volumes of a 0.25 μg. per ml. acetone solution were pipetted into a series of the flasks. The acetone was evaporated in an air stream, and the remainder of the procedure was carried out as in the analysis of forage. Ten microliters of each standard was injected into the column. Peak height in cm. was plotted against μg. of herbicide.

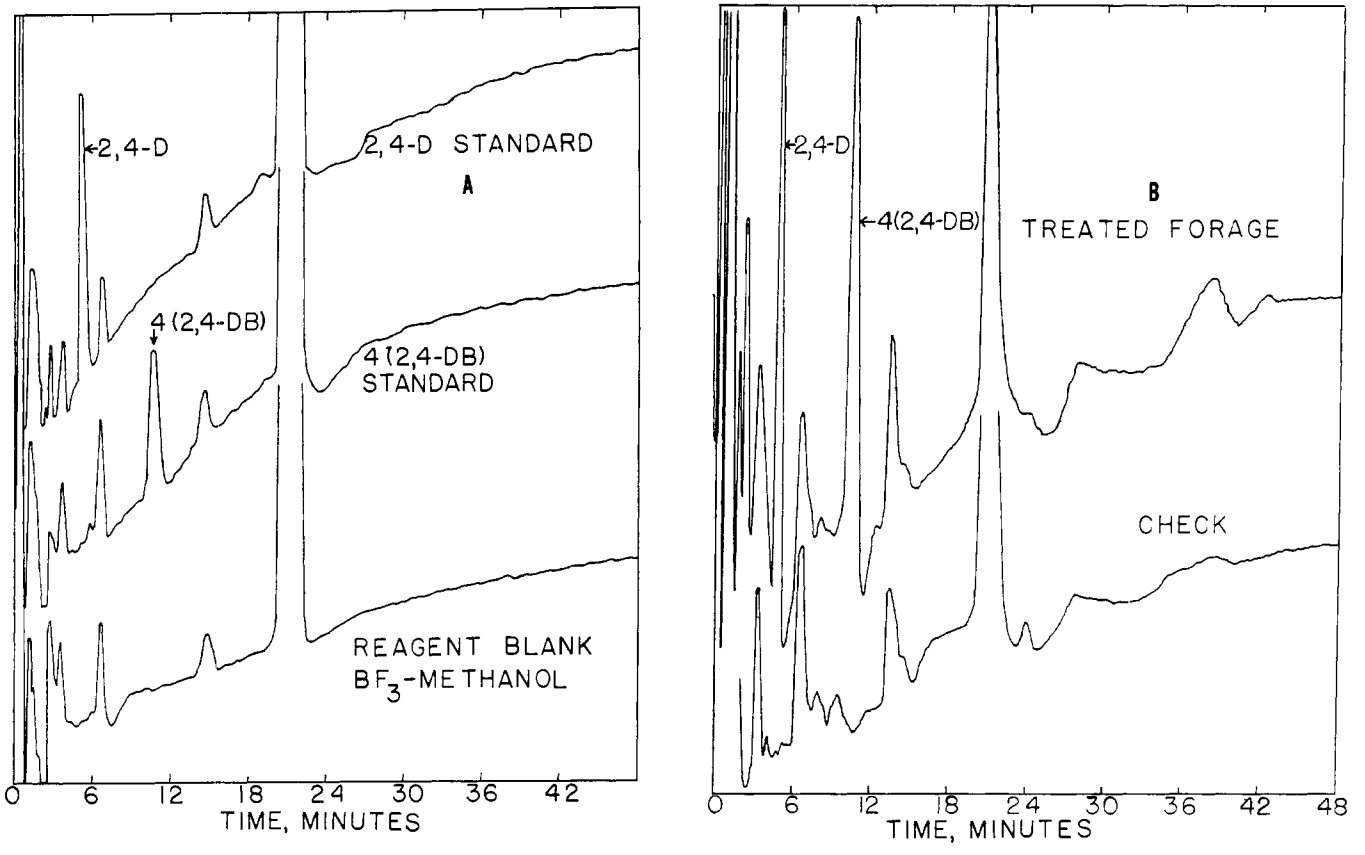

Figure 1. Chromatograms of (A) esterified standard solutions of 4(2,4-DB) (0.002 μg.) and 2,4-D (0.001 μg.) and the BF_3-methanol reagent blank and (B) untreated forage and forage sampled 43 days after treatment with 3 pounds of 4(2,4-DB) per acre

Results and Discussion

Figure 1*A* shows the chromatograms of esterified 4(2,4-DB) and 2,4-D and the BF_3-methanol reagent blank. The peaks appearing in the reagent blank were probably due to high boiling impurities in the solvents. Technical grade acetone and distilled hexane were used for extraction. The large peak with a retention time of about 20 minutes was due to an impurity in the C.P. methanol used in the preparation of the BF_3 reagent. The chromatograms of untreated birdsfoot trefoil–timothy grass and the sample treated with 3 pounds per acre of 4(2,4-DB) and sampled 43 days later are shown in Figure 1*B*.

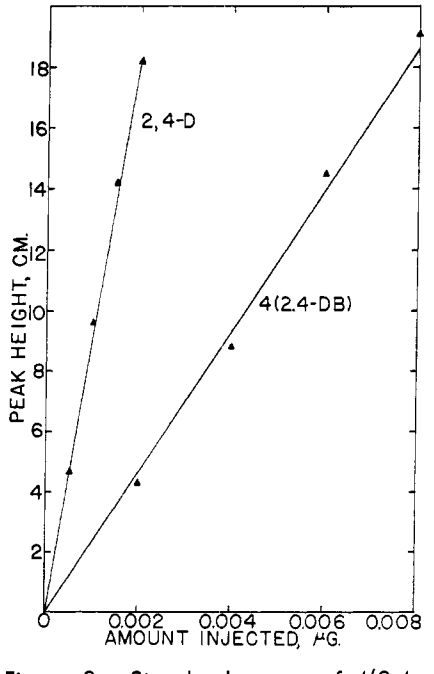

Figure 2. Standard curves of 4(2,4-DB) and 2,4-D

The recoveries of 4(2,4-DB) and 2,4-D added to untreated birdsfoot trefoil–timothy forage are shown in Table I. The method is sensitive to about 0.2 p.p.m. of 4(2,4-DB) and 0.1 p.p.m. of 2,4-D. These concentrations would yield peak heights equal to about a 5% full-scale deflection when injecting 10 μl. of the sample. The sensitivity obtained using the electron affinity detector for compounds having chlorines on a benzene ring is lower than with chlorines on a cyclohexane ring (lindane) or on a 7-member ring (dieldrin, etc.) (*4*). This is presumably due to the electronegativity of chlorine having been lowered by the available pi-electrons in benzene. Conversely, the addition of the methoxy ester group increases electron affinity.

Figure 2 shows typical standard curves for the herbicides in which peak height in centimeters is plotted against micrograms injected.

Table I. Recovery of 4(2,4-DB) and 2,4-D Added to Birdsfoot Trefoil–Timothy Grass Forage

Amount Added, P.P.M.	*Recovery, %*
	4(2,4-DB)
0.8	85, 90
1.6	93
4.0	90, 92, 96, 94
	2,4-D
0.4	102, 90, 92
3.0	99, 103, 102

The disappearance of 4(2,4-DB) in the birdsfoot trefoil–timothy forage at both rates of application is shown in Figure 3. Rainfall is indicated by the broken vertical lines. The June 22 rainfall occurred immediately after the 1.5 pounds per acre sample was taken. The 3 pounds per acre sample was taken after the rain. Therefore the 3-pound, June 23 sample showed a 30% loss of residue, whereas the 1.5-pound sample showed its sharpest decrease (29%) on June 24.

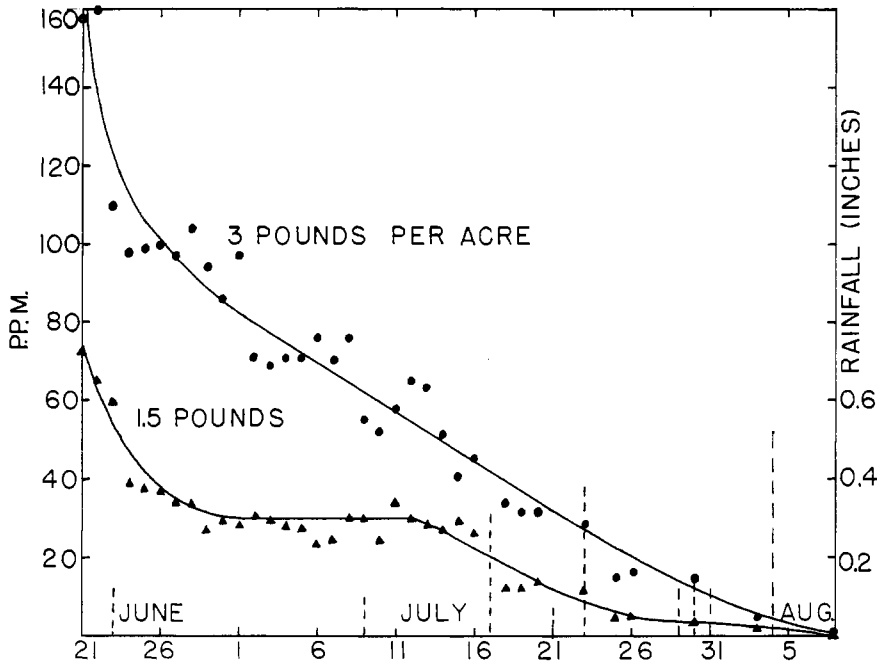

Figure 3. Disappearance of 4(2,4-DB) in birdsfoot trefoil–timothy grass forage treated at 1.5 and 3 pounds per acre

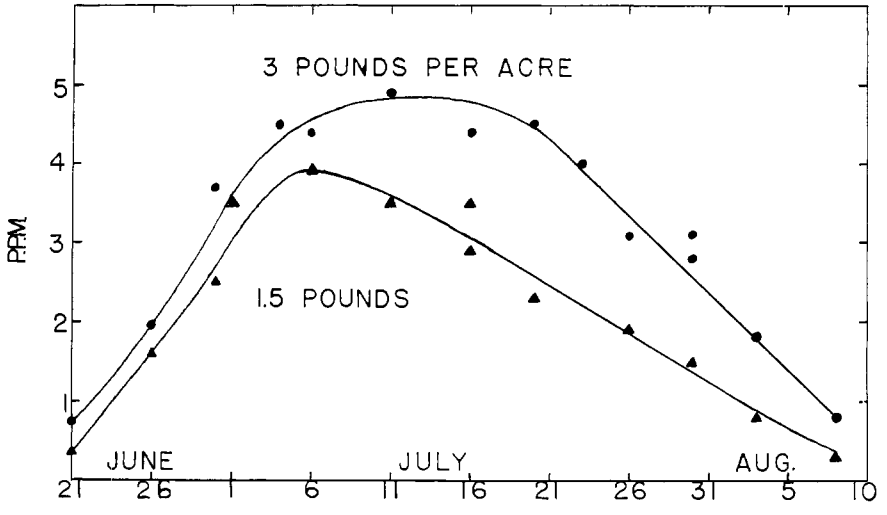

Figure 4. Formation and disappearance of 2,4-D in 4(2,4-DB)-treated forage

Glastonbury, Stevenson, and Ball (*1*), using infrared spectroscopic and isotope dilution methods, showed a similar disappearance of 4(2-methyl-4-chlorophenoxy)butyric acid (applied as the sodium salt) in peas. The residues of the herbicide on August 8 (48 days after treatment) at the 1.5- and 3-pound rates were 0.32 and 0.80 p.p.m., respectively.

Figure 4 shows the formation and persistence of 2,4-D with time after treatment of the forage at both rates with 4(2,4-DB). In the 3-pound treatment, 2,4-D reached its maximum concentration of 4.9 p.p.m. in the plant on about July 11 or 20 days after treatment. Also, although about 250% more 4(2,4-DB) was present in the forage treated at 3 pounds per acre, the maximum concentration of 2,4-D was only about 125% higher than in the 1.5-pound treatment. This may indicate that there is a definite threshold value for 2,4-D formed in the plants even though large excesses of 4(2,4-DB) may be present. It may be that further increases in 2,4-D concentration are limited by transport mechanisms in the plant which possibly bring 4(2,4-DB) to the proper site for beta oxidation to 2,4-D.

Acknowledgment

The authors thank S. N. Fertig and A. Furrer for applying the herbicide to the forage.

Literature Cited

(1) Glastonbury, H. A., Stevenson, M. D., Ball, R. W. E., *Weeds* **7,** 362 (1959).
(2) Goodwin, E. S., Goulden, R., Reynolds, J. G., *Analyst* **86,** 697 (1961).
(3) Goodwin, E. S., Goulden, R., Richardson, A., Reynolds, J. G., *Chem. Ind. London* **1960,** p. 1220.
(4) Lovelock, J. E., *Anal. Chem.* **33,** 162 (1961).
(5) Metcalfe, L. D., Schmitz, A. A., "Chromatography Application Methods Bulletin WCA 1," Barber-Colman Co., Rockford, Ill., Sept. 22, 1960.

Received for review August 16, 1962. Accepted November 6, 1962.