dustry as a fodder supplement for beef cattle, thereby improving meat quality and increasing quantity in less time with less food.

References

1. SMALL, J. K. *The Manual of Southeastern Flora.* New York : Publ. by author (1933).
2. FEURT, S. D., and FOX, L. E. *J. Am. Pharm. Assoc.*, **41**, 453 (1952).
3. PINCUS, G., and THIMANN, K. V. *The Hormones,* Vol. 2. New York : Academic Press (1950).
4. WEICHERT, C. K., and KORRIGAN, S. J. *Endocrinology,* **5**, 741 (1942).
5. ZONDEK, B. *Lancet,* **10**, 10 (1936).
6. SLEETH, R. B. Agricultural Dept., University of Florida, personal communication.
7. ANDREWS, F. N., BEESON, W. M., and JOHNSON, F. D. *J. Animal Sci.*, **9**, 677 (1950).
8. WEBBER, M. G., LAUTER, W. M., and FOOTE, P. A. *J. Am. Pharm. Assoc.*, **41**, 230 (1952).
9. HALLIGAN, J. E. *J. Ind. Eng. Chem.*, **1**, 206 (1909).
10. PICKETT, J. M. *Florida Expt. Sta. Bull. II* (1890).
11. TRENCH, R. B., and ABBOTT, O. D. *Florida Agr. Expt. Sta. Tech. Bull. No. 444* (1948).

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2,4-D Affects Phosphorus Metabolism

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A preliminary experiment in which *Commelina* sp. and *Xanthosoma* sp. were analyzed 24 hr, and 1 wk after being treated with 2,4-D (2,4-dichlorophenoxyacetic acid) showed that the percentage of water-soluble phosphorus in treated plants was consistently higher than in untreated plants. The following experiment was carried out to obtain additional information on the effect of 2,4-D on phosphorus metabolism.

A prepared field was divided into 12 plots each 52×24 ft and planted to a variety of white beans, Blanca Bonita (P.R. 1632). The experimental design consisted of 3 treatments each replicated 4 times in randomized blocks.

When the plants were about 15 in. high and had started to set fruit 1 plot in each of the 4 replications was sprayed with 0.1% aqueous solution of sodium 2,4-D. The plants in another plot of each replication were uprooted at the same time and left lying on the ground to die gradually. The third plot in each replication was left as a control. Two rows of unsprayed or undisturbed plants were left as borders around each plot. The treatments were started at 6 A.M., and samples of 100 plants were taken from each replication of all treatments at 4, 10, 24, and 48 hr and 1 wk after treatment.

The leaves, stems, and roots were separated, fresh and dry weights obtained, and a composite sample of 300 g of dry powdered tissue from each replication was analyzed for inorganic phosphorus. Aliquots of a hot water extract of the dry tissues were clarified with 0.5 g charcoal and used for inorganic phosphorus determination as described by Truog and Meyer (*1*).

Four hours after treatment the sprayed plants showed epinasty and other 2,4-D effects. Ten hours after treatment many of the leaves had curled, and the plants were becoming recumbent. The next morning the plants were somewhat chlorotic and the distortion had increased. The following day many of the leaves had developed necrotic spots. One week after treatment the leaves on most sprayed plants had withered and those that adhered were very chlorotic and sickly in appearance.

The uprooted plants remained fresh for the first day but after that they deteriorated so rapidly that by the end of the week it was not possible to obtain leaf samples.

Figure 1 shows the fluctuations of inorganic phosphorus in the leaves, stems, and roots, respectively, of treated, check, and uprooted plants.

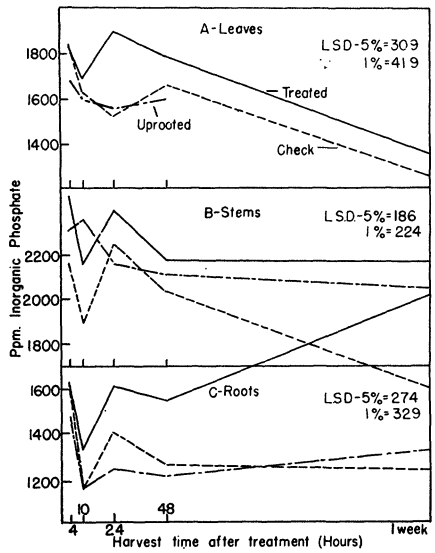

FIG. 1. Levels of inorganic phosphorus (ppm of dry matter) in bean plants analyzed at various intervals after treatment with 0.1% solution of sodium 2,4-D.

The data obtained at each sampling period, expressed as parts of inorganic phosphorus per million parts of dry material, was analyzed statistically by the analysis of variance and the least significant differences between treatments determined.

Four hours after treatment there was no appreciable difference in the amount of inorganic phosphorus in the leaves and roots of treated and check plants, but the stems of treated plants had a significantly higher amount than the checks. The uprooted plants had somewhat less inorganic P than the treated plants in all 3 organs. Ten hours after treatment inorganic P had dropped in all organs of all treatments except in the stems of uprooted plants, where it was somewhat higher; but in roots, stems, and leaves of treated plants it was higher and significantly more so in the roots and stems than in the corresponding organs of check plants. The samples taken 24 hr after treatment showed a sharp rise in the level of inorganic P in roots,

[1] Administered by the Office of Experiment Stations, Agricultural Research Administration, USDA.

stems, and leaves of treated plants; the level in the roots and leaves was significantly higher than in the checks. There was also a rise in the inorganic phosphorus content of stems and roots of check plants, but it was less than in treated plants. The leaves of check plants showed no increase as did the leaves of treated plants in this phosphorus fraction, indicating that the most pronounced effect of 2,4-D on phosphorus metabolism occurred in the leaves at this time.

Forty-eight hours after treatment the level of inorganic P in leaves, stems, and particularly roots of treated plants was significantly higher than in check plants. In the roots, stems, and leaves of uprooted plants it was about the same as in the check plants. One week after treatment inorganic P in the roots of treated plants had increased significantly and this coincided with a sharp decline in the leaves, indicating that it may have been translocated from the leaves to the roots. There was practically no change in the inorganic phosphorus fraction in treated stems. Although by this time the level in the leaves and stems of check plants also declined, there was no corresponding increase in the check roots as there was in the treated roots.

Inorganic P in leaves and stems of treated plants fluctuated in most instances like that in the check plants, but this fraction was consistent, and at most sampling dates, except the first, significantly higher in roots, stems, and leaves of treated plants.

This experiment provides a clue to the mode of action of 2,4-D, e.g., it may inhibit or interrupt the phosphate metabolism in the plant. These data and the fact that very small amounts of 2,4-D produce drastic effects suggest that 2,4-D may inhibit or poison the enzyme or system responsible for the hydrolysis or synthesis of the high energy phosphates.

Reference

1. TRUOG, and MEYER. *Ind. Eng. Chem. Anal. Ed.*, **1**, 136 (1929).

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Colorimetric Method for Determination of Aureomycin, Carbomycin, Erythromycin, and Terramycin in Aqueous Solution

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We have observed that the acid hydrolyzates of Aureomycin (*1*), carbomycin (*2*), erythromycin (*3*), and Terramycin (*4*) react with the arsenomolybdate reagent to produce blue colored complexes. The optical density of the color formed has been found to be a function of the quantity of antibiotic present. Satisfactory results have been obtained with the following procedure.

Aliquots containing from 10 to 40 μg of antibiotic are added to colorimeter tubes and the solution evaporated to dryness using an air jet. Two milliliters of $6N$ H_2SO_4 and 1 ml of arsenomolybdate reagent (Nelson's [*5*] reagent diluted with 2 parts of distilled water) are added. The tubes are plugged with loose fitting corks and placed in a boiling water bath. After a 15-min heating period the tubes are cooled to room temperature and the contents diluted with 5 ml of distilled water. Color intensity is determined using a photoelectric colorimeter equipped with 660 mμ filter. A series of tubes containing known quantities of the antibiotic are prepared and treated simultaneously with the tubes containing unknown quantities of the antibiotic. The values obtained with this series of known solution are used to calculate the constants of Beers' law and to standardize the determinations.

The sensitivity of the method varies somewhat with the particular antibiotic under consideration. The practical working range for Terramycin and Aureomycin is from 2 to 40 μg/tube; for erythromycin it is 5–80 μg/tube; and for carbomycin it is 10–160 μg/tube. Smaller quantities may be determined if only 1 ml of distilled water is added after the heating period, and micro cells are used to determine the optical densities. Apparently the hydrolysis with 6 N acid is necessary to obtain maximum sensitivity of the method, and use of more dilute acid resulted in reduced sensitivity. Only Terramycin will reduce the arsenomolybdate reagent without a preliminary hydrolysis, and in this instance the working range has been found to be from 20 to 160 μg/tube. Some of the data collected in analyzing aqueous solutions containing known quantities of Terramycin are summarized in Table 1.

TABLE 1

ANALYSIS OF SOLUTIONS FOR TERRAMYCIN CONTENT

Solution	Terramycin added* μg/ml	Terramycin found† μg/ml
Distilled water	0	0
	10	9.7; 9.9; 10.2
	30	29.8; 30.7; 30.7
	100	98.6; 99.7; 101.5
2% Glucose	0	0
	10	9.5; 9.7; 10.0
	30	27.7; 30.6; 30.6
2% Starch	0	0
	10	9.1; 9.7; 9.7
	30	28.8; 29.0; 29.6

* Terramycin hydrochloride was used in these studies. All analyses are presented in terms of the free base.

† Antibiotic extracted from aqueous solution with methyl isobutyl ketone.

This method cannot be applied directly to solutions containing carbohydrates and other substances which react when heated with the arsenomolybdate reagent. These four antibiotics may be separated from carbohydrates by extraction from aqueous solution (pH 7.2) into chloroform, amylacetate, n-butanol and methylisobutyl ketone. All the antibiotic has been recovered in the solvent phase when equal volumes of the solvent and aqueous solution have been used.