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SHORT COMMUNICATION

THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE RUTIN CONTENT OF TOMATO PLANTS

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Abstract—A paper chromatographic method has been modified to use fluorometry for the quantitative determination of rutin in tomato plants. Using this method, quantitative analyses were made periodically over a period of 12 days on the rutin content of stems and leaves of control as well as of 2,4-D-treated tomato plants. By the twelfth day, the rutin content of the leaves of the control plants was more than 4 times as great as that of the leaves from 2,4-D-treated plants, and the rutin content of the stems of the control plants was more than 10 times that of the stems from 2,4-D-treated tomato plants.

INTRODUCTION

RUTIN (3-rutinoside of quercetin, 3,3′,4′,5,7-pentahydroxyflavone) has been previously reported[1–3] to be present in the stems and leaves of several varieties of tomato plants. No previous report, however, has been made on the rutin content of tomato or other plants after their having been sprayed with 2,4-dichlorophenoxyacetic acid (2,4-D). Using a paper chromatographic-fluorometric procedure for the quantitative analysis of rutin, a comparison of the amounts of rutin in stems and leaves of 2,4-D-treated tomato plants with those in control plants has been made periodically up to 12 days following treatment.

RESULTS AND DISCUSSION

The average of the quantitative duplicate determinations of rutin in leaves and stems of tomato plants sprayed with 2,4-D and also of control tomato plants are given in Table 1. No rutin was found in the roots of either the treated or the control plants.

Previous studies by Dieterman *et al.*[4] have shown that there is a significant increase in the amounts of scopoletin (6-methoxy-7-hydroxy-coumarin) and scopolin (scopoletin-7-glucoside) in tobacco, *Nicotiana tabacum*, after these plants had been sprayed with 2,4-D. The present investigation on rutin has revealed a significantly lower amount of rutin, in comparison with control plants, in the stems and leaves of tomato plants analysed four or more days after being sprayed with 2,4-D. The differential in rutin content between control and 2,4-D-treated tomato plants increased at each harvest time from the fourth through the twelfth day, which was the last day investigated. The processes by which 2,4-D produces these effects still need elucidation.

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[1] B. K. BLOUNT, *J. Chem. Soc.* **136**, 1528 (1933).
[2] T. D. FONTAINE, R. MA, J. B. POOLE, W. L. PORTER and J. NAGHSKI, *Arch. Biochem. Biophys.* **15**, 89 (1947).
[3] MING-AN WU and R. C. BURRELL, *Arch. Biochem. Biophys.* **74**, 114 (1958).
[4] L. J. DIETERMAN, C-Y. LIN, L. ROHRBAUGH, V. THIESFELD and S. H. WENDER, *Analyt. Biochem.* **9**, 139 (1964).

In the studies on stems, both the control and the 2,4-D-treated tomato plants showed a decrease in rutin content on days 1 and 2 after the time of spraying. In leaves of the control there was a decrease in the rutin content on day 1 over the amount on day 0; thereafter, there was a steady increase through the end of the experiment on day 12. In leaves of the sprayed plants, there was some increase in the rutin content on days 1 and 2, and then a gradual decrease to a level only slightly above that found on day 0. The first two days after the spraying were unusually cool and dark days for the month of May in Oklahoma, and these conditions may possibly have had a significant effect on the rutin content of plants harvested at this time. Proper evaluation of these effects, however, cannot be made without additional experimental studies. Some studies have been made by Griffith, Krewson, and Naghski[5] on the effects of age and time of planting on the rutin content of buckwheat, but we know of no such work on tomato plants.

TABLE 1. RUTIN CONCENTRATION IN UNTREATED AND IN 2,4-D-TREATED TOMATO PLANTS

Days from start	Rutin in (μg/g fresh wt.)			
	Leaves		Stems	
	Not treated	Treated	Not treated	Treated
0	94	99	22	14
1	85	130	16	13
2	95	140	10	6
3	165	136	16	13
5	190	110	35	18
7	335	125	32	10
9	360	110	74	8
12	580	125	100	8

MATERIALS AND METHODS

Growth and Preparation of Tomato Samples

Seed from tomato, *Lycopersicon esculentum* Mill., variety Marglobe were planted in soil in the greenhouse on April 20, 1964. After about two weeks, uniform seedlings were selected and transplanted to soil in glazed crocks. On May 18, 1964, 33 plants were sprayed over their entire surfaces with a solution containing 1 g of 2,4-D and 5 g of Carbowax 1500 per l. and adjusted to pH 7 with NH_4OH. The control group included 33 plants which were not sprayed. The plants were 28 days old from time of seeding and approximately 10 in. high at the time of spraying, and the greenhouse temperature was 27°. Three sprayed and three control plants were harvested immediately after the time of spraying and at 1, 2, 3, 5, 7, 9, and 12 days thereafter.

The plants were divided into leaf, stem, and root portions immediately after harvesting, and each portion was homogenized with boiling methanol in a Waring blendor. The mixture was filtered, and the residue mixed again with fresh boiling methanol for 10 min. After filtration, extraction of the residue was completed with methanol in a Soxhlet extractor.

[5] J. Q. GRIFFITH, C. F. KREWSON and J. NAGHSKI, In *Rutin and Related Flavonoids*, p. 37. Mack, Easton, Pa. (1955).

The extracts of each portion were combined and concentrated *in vacuo* with a rotary evaporator.

Isolation and Identification of Rutin

Aliquots of the methanol concentrates were streaked on sheets of Whatman No. 1 chromatography paper. For development, *n*-butyl alcohol:acetic acid:water (6:1:2, v/v, BAW) was used. The zone containing rutin (R_f 0·42–0·47) was cut out and re-run using first aqueous 2% acetic acid (HOAc) (rutin R_f 0·33–0·39) and second, methyl isobutyl ketone:formic acid:water (14:3:2, v/v, KFW). Repetition of the last procedure usually resulted in a compound which was chromatographically pure, but in some cases all three steps were repeated. The compound co-chromatographed with authentic rutin in BAW, HOAc, and KFW, with R_f values of 0·44, 0·37, and 0·20, respectively. The isolated rutin was mixed with aqueous 1% hydrochloric acid and the mixture boiled for 10 min. The aglycone was extracted from the mixture with ethyl acetate. The chromatographic behavior of the aglycone and authentic quercetin were the same in all solvents tested. From the aqueous residue, chromatograms were prepared and developed in BAW and *n*-butyl alcohol:benzene:pyridine:water (5:1:3:3, v/v). The presence of glucose and rhamnose in the aqueous extract was established by co-chromatography with authentic glucose and rhamnose, using aniline and oxalic acid as the locating spray.[6]

Quantitative Determination of Rutin

The quantitative procedure used was based in part on those previously described by Gage and Wender.[7] and by Yang *et al.*[8] A major modification involved the use of fluorometry rather than spectrophotometry for quantitative determination of the rutin separated by paper chromatography.

Aliquots of the methanol concentrates of the tomato extracts were spotted on Whatman No. 1 papers. Two dimensional chromatograms were developed with BAW as the first and HOAc as the second solvent. The chromatograms were dried overnight. A portion of the chromatogram paper, 2·5 × 2·5 in., containing the rutin zone, was cut out. This piece of paper was cut into approximately fifty pieces and subsequently mixed with a methanolic 1% aluminum chloride solution. The mixture was shaken for one hour, then filtered through glass wool. The filtrate was brought to volume with methanolic 1% aluminum chloride solution. The relative fluorescence intensity of the rutin-aluminum chloride mixture was measured with a Turner fluorometer. The concentration of the rutin was then determined from an internal standard reference curve, using known weights of authentic rutin carried through identical steps (a straight line was obtained with between 6 and 22 μg).

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