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ETHEPHON EFFECT ON PROTEIN SYNTHESIS IN FIG FRUITS

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Key Word Index—*Ficus carica*; Moraceae; fig; ethephon; 2-chloroethylphosphonic acid; ethylene; protein synthesis; fruit ripening.

Abstract—Ethephon (2-chloroethylphosphonic acid) applied to fig fruits increased the incorporation of ^{14}C-phenylalanine into protein in extracts of the fruit. A 20% increase in the amount of ^{14}C-phenylalanine incorporated *in vitro* was detected 24 hr after treatment. A peak (36% increase over control) was reached 2 days later and was followed by a gradual decline as fruits ripened. The increase in protein synthetic activity detected with the fig fruit system following treatment with ethephon could be attributed primarily to the components of the pH 5 enzyme fraction and to a lesser extent to ribosomes. Ethylene gas released from ethephon in fig fruit tissue is thought to be responsible for the increased activity.

INTRODUCTION

THE PHASIC growth pattern characteristic of fig fruit[1] provides a system that can be used to investigate cellular phenomena associated with, and which possibly affect, growth and development. Furthermore, the growth pattern of the fig fruit was shown to be altered by the application of plant growth regulators. For example, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) applied at the beginning of Period II (the phase of slow growth that separates Periods I and III—the rapid growth phases) resulted in continued rapid growth and considerably earlier ripening.[2] Ethylene[1] and ethephon[3] application during the second half of Period II, promoted growth and the initiation of ripening (Period III).

The response of the fig fruit to such treatments could be of considerable value in investigating cellular control mechanisms at the molecular level. Ribosomes[4] and RNA* were isolated from fig fruit tissue and their properties characterized. In addition, a cell-free system capable of incorporating amino acids into protein (*in vitro*) was prepared from the same tissue.[5] Since ethylene was shown to enhance *in vivo* synthesis of ribosomes, RNA and protein in fig fruits,[6] the present study was undertaken to confirm that effect in a cell-free

* Marei and Romani, in preparation.

[1] N. MAREI and J. C. CRANE, *Plant Physiol.* **48,** 249 (1971).
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[4] N. MAREI and R. J. ROMANI, *Biochem. Biophys. Acta* **247,** 280 (1971).
[5] N. MAREI, A. I. GADALLAH and W. W. KILGORE, *Phytochem.* **11,** 529 (1972).
[6] N. MAREI and R. J. ROMANI, *Plant Physiol.* **48,** 806 (1971).

system which removes possible effects of permeability and pool-size factors. Ethephon, a compound known to decompose in plant tissue to release ethylene,[7,8] was used as the source for the gas instead of ethylene *per se*.

RESULTS AND DISCUSSION

The physiological and biochemical responses of the fruits to the 3 ethephon treatments during the second half of Period II were very similar, however, maximum responses were obtained with the 17 June treatment. Therefore, only data obtained from that treatment are presented here. Ethephon treatment stimulated fruit growth considerably and enhanced ripening which occurred 2 weeks earlier than in the controls (Fig. 1). The ultimate diameter and edible qualities of ethephon-ripened fruits were indistinguishable from the untreated ones.

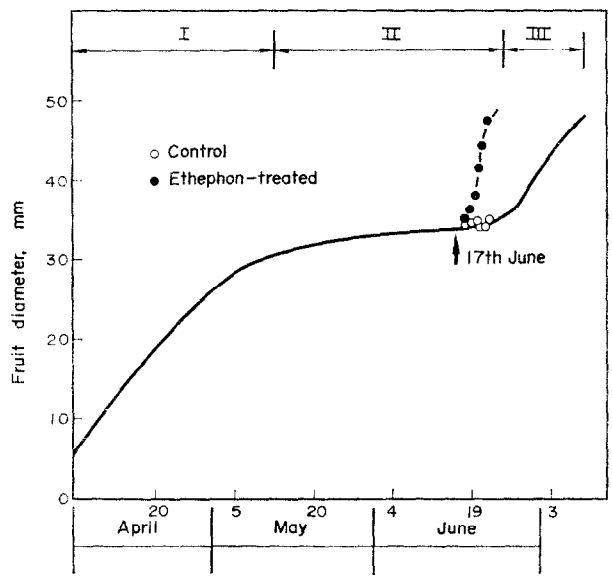

FIG. 1. EFFECT OF ETHEPHON (200 μl/l.) APPLIED 17 JUNE ON THE GROWTH OF FIRST-CROP FIG FRUITS. ROMAN NUMERALS INDICATE THE 3 GROWTH PHASES CHARACTERISTIC OF FIG FRUITS.

Following ethephon application, treated and control fruits were sampled daily for 6 days to assess the relative activity of their cell-free systems in synthesizing protein *in vitro*. When ribosomes and pH 5 enzyme fractions were prepared from treated fruit tissue 1 day after application, a 20% increase in the amount of ^{14}C-phenylalanine incorporated into protein was detected (Table 1). A peak in the incorporation activity (36% over control) occurred with the cell-free system prepared from treated fruits sampled 3 days after treatment. A decline in protein synthetic activity occurred thereafter, and reached values below those of the control sampled on the 5th and 6th day.

[7] H. L. WARNER and A. C. LEOPOLD, *Plant Physiol.* **44,** 156 (1969).
[8] S. F. YANG, *Plant Physiol.* **44,** 1203 (1969).

To determine whether the increase in protein synthetic activity *in vitro* was attributable to ribosomes and/or the pH 5 enzyme fraction, ribosomes from untreated tissue were incubated with pH 5 enzyme fraction prepared from ethephon-treated fruits; and vice versa in another series of incubations. In both cases, an increase in ^{14}C-phenylalanine incorporation occurred when the cell-free systems were prepared 1 day after treatment (Table 1). A peak in incorporation activity occurred 3 days after treatment that was followed by a decline to values below those of the control sampled on the 5th and 6th day (Table 1). Although the incorporation activity data assumed a pattern similar to that formed when the source of both ribosomes and pH 5 enzyme fractions was ethephon-treated fruits, none of them reached its magnitude. Furthermore, it is clear that the increase in protein synthetic activity can be attributed to both subcellular fractions, although more so to the pH 5 enzyme components (Table 1).

TABLE 1. EFFECT OF ETHEPHON ON PROTEIN SYNTHESIS *in vitro* BY A CELL-FREE SYSTEM PREPARED FROM FIG FRUITS

Days after treatment	Specific activity (μmol ^{14}C-phenylalanine/mg RNA)			
	Control	T*-ribosome + T-supernatant	C*-ribosome + T-supernatant	T-ribosome + C-supernatant
0	43·2	—	—	—
1	45·4	54·5 (20)	53·7 (18)	47·7 (5)
2	42·8	54·4 (27)	51·1 (19)	47·1 (10)
3	44·4	60·4 (36)	53·3 (20)	50·2 (13)
4	46·1	57·6 (25)	50·7 (10)	47·9 (4)
5	45·8	41·8	44·7	44·0
6	46·6	38·2	40·5	39·1

*T—From ethephon-treated fig fruit tissue; C—from untreated tissue.

Ethephon (200 μl/l.) was applied to fruit-bearing branches in the orchard on 17 June, 1971 (see Fig. 1). The incubation medium and procedure are described in the text. Figures in parentheses indicate % increase over control.

The data presented here suggest that ethephon caused an activation of the protein synthetic machinery in fig fruit tissue. Since it is known that ethephon decomposes in plant tissue to release ethylene[7,8] and that identical responses were obtained when fig fruits are treated with ethylene or ethephon,[1,3] it may be assumed that ethylene liberated from the chemical is responsible for the effect on protein synthesis. Such an assumption seems to be justified in view of the finding that ethylene gas (5 μl/l.) enhanced synthesis of ribosomes, RNA and protein *in vivo* in fig fruit tissue.[6] In addition, the enhancement of protein biosynthesis was found to reach a peak 3–4 days from the inception of ethylene treatment.[6] In other words, the *in vitro* assessment of protein synthetic machinery examined in the present study reflected the changes occurring in the parent tissue *in vivo*. Furthermore, the results presented here substantiate an earlier conclusion[6] that ethylene-stimulated protein synthesis was not due to increased cellular permeability and greater availability of precursors. These findings may serve as additional evidence supporting the hypothesis that ethylene could be promoting fig fruit ripening by enhancing biosynthesis of specific proteins.[6] The decline in protein synthesis 4 days after treatment may represent the cessation of the synthesis of proteins required to trigger ripening.

It is evident from the current study that although the increase in ^{14}C-phenylalanine incorporation *in vitro* was associated with both the pH 5 enzyme and ribosomal fractions, the components of the former seemed to be more responsible for the increase. Since the ribosomes were not washed, however, the possibility of their contamination with pH 5 fraction components exists.[9] This may have contributed to the increased protein synthesis *in vitro* noted when ribosomes isolated from ethephon-treated tissue were incubated with pH 5 enzyme fraction from control fruits. However, an increase in the capacity of ribosomes from treated tissue to incorporate amino acids should not be overlooked. In this case, the stimulated ribosomal activity could have been due to increased synthesis of new ribosomes and/or conversion of monomers to polysomes. In view of earlier results,[6] we favor the former possibility. A definite effect of ethylene on the components of pH 5 enzyme fraction, on the other hand, could be discerned from our study.

Although earlier[6] and present results indicate a significant enhancement in the rates of synthesis of ribosomes, RNA and protein in fig fruits treated with ethylene, more work is needed to determine whether the mechanism for protein synthesis is the primary site of action by which ethylene promotes growth and ripening of the fig fruit.

EXPERIMENTAL

The growth curve of first-crop fig (*Ficus carica* L., cv. Mission) fruits (1971) was used as a reference in timing the application of ethephon. This was done by periodical measurement of several fruits on 4 trees.[1] During the second half of growth period II, fruit-bearing branches were sprayed with 200 μl/l. of ethephon to the point of run-off. Three applications were made at weekly intervals starting 3 June. Treated and control fruits were sampled daily following treatment, brought to the laboratory in ice and stored at $-60°$. Each sample consisted of 4–5 fruits. The ripening of ethephon-treated fruits (usually 6–8 days after treatment) marked the termination of a single experiment.

Ribosomes were isolated and the pH 5 enzyme fraction prepared from fruits according to methods described earlier.[4,5] The incubation medium and the procedures for amino acid incorporation (*in vitro*) as well as the precipitation of labelled protein and assay of radioactivity were all carried out as described previously.[5] Polyuridylic acid (50 μg/ml) and Mg^{2+} (12 mM) were incorporated in the incubation medium, and the equivalent of 400 μg RNA from both the ribosomal suspension and the pH 5 fraction were added. The amount of RNA in both preparations was determined by the orcinol method.[10] One μCi of ^{14}C-L-phenylalanine (50 mCi/mmol) was added to each of the incubation media along with 0·1 mmol of each of the 19 essential amino acids. The incubation period was 40 min at 37°.

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