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United States - Israel Binational Agricultural Research and Development Fund

FINAL REPORT

PROJECT No. I-61-79

Production, Regulation and Mode of Action of Ethylene in Fruit Ripening, Senescence, and Decay

E. Chalutz, Y. Fuchs, J.D. Anderson

1984

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BARD TECHNICAL REPORTS

BARD No. of Proposal I-61-79

Title of Proposed Research Production, Regulation and Mode of Action of Ethylene in Fruit Ripening, Senescence and Decay.

Name(s) of investigator(s):

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Cooperating investigator(s) Y. Fuchs, J.D. Anderson

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Cooperating institution(s) Plant Hormone Laboratory, BARC, ARS, USDA
Beltsville, Md 20705, U.S.A.

Project's starting date Sept. 20, 1979

Type of Report: initial ☐ Comprehensive ☐ Final ☒

Signature

Principal Investigator

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Abstract

The research work of this BARD project proceeded along two main lines: The first one comprised of studies related to biosynthesis of ethylene. We elucidated some properties and the membrane association of the ethylene forming enzyme, the effect of some drugs - antagonists of calmodulin - on ACC synthase activity and described the role of inorganic phosphate in the biosynthesis of ethylene in higher plants and microorganisms.

The second main research direction centered on the role of ethylene in host-pathogen interactions. We studied the effect of fungal infection on inhibition of ethylene-induced PAL activity in citrus fruit and on the ethylene producing machinery of the host. The contribution of host and pathogen to the ethylene produced was determined in P. digitatum - infected citrus fruit. We also demonstrated a rapid induction of ethylene biosynthesis in tobacco leaves when leaf discs were incubated with cell-wall digesting enzymes and demonstrated the stimulation of this phenomenon by ethylene, in an autocatalytic manner. The effect of wounding in relation to avocado fruit ripening was also studied. The results obtained have thus deepened our knowledge of these two main research areas.

This BARD project also promoted close, fruitful and productive scientific cooperation between the American and Israeli scientists involved.

Body of the report

The research results of this project can be summarized under two main headings as outlined in the proposal: Biosynthesis and mode of action of ethylene, and the role of ethylene in host-pathogen interactions.

The enclosed papers and manuscripts, which comprise the body of the report, have therefore been arranged accordingly in two main groups.

Evaluation of research achievement with respect to original research proposal

Of the three main research objectives outlined in the proposal, viz. the elucidation of the biosynthesis and mode of action of ethylene, the determination of the role of ethylene in host-pathogen interactions and the development of techniques for suppression or accelerating ethylene production and action to control the ripening process, the research achievements are related mainly to the first two objectives. As to the third objective, we did find a process in which rapid and massive ethylene production occurs but this has not yet been applied for the control of fruit or vegetable ripening.

Description and results of cooperation

Throughout the time span of this project, several visits of the participating scientists at the laboratories of their cooperating investigators took place. These consisted of short visits (1 to 2 weeks) intended for the exchange of information; planning of experiments, development of new research procedures or the summarizing the work and writing reports. In addition, the principal investigator (E. Chalutz) spent an extended period (Sabbatical year) at the laboratory of the cooperating investigator.

Another scientist who cooperated in this project, A.K. Mattoo, had previously spent an extended period (about 2 years) working partly with scientists at the laboratory in Israel and then joined the U.S. laboratory where he has been working, among other projects, on this BARD project, for the past 3 years. Partial salary support for Dr. Mattoo was from this BARD project.

Throughout the duration of the project, special chemicals such as inhibitors and fungal isolates were exchanged between the cooperating laboratories.

On the whole, this project promoted close collaboration between the two research groups with many joint publications and a new BARD proposal submitted last fall.

On January 18, 1982, during the course of this project, M. Lieberman, the cooperating American investigator passed away. His successor as Chief of the Plant Hormone Laboratory BARC, J.D. Anderson, joined in the project and promoted further the research work and the close cooperation.

As a result of the initiative of all the scientists involved in this BARD project (and with the help of others), a special International Symposium on Ethylene, dedicated to the memory of M. Lieberman, was held in Israel in January 1984. This successful event reflected the close cooperation between American and Israeli scientists in the field of ethylene, a research area which has been promoted by BARD.

Thesis

1. Kapulnik, Esther (1981). Production of ethylene by two isolates of the fungus Penicillium digitatum. M.Sc. Thesis. Submitted to the Faculty of Agriculture of the Hebrew University of Jerusalem, Israel.
2. Achilea, Oded (To be submitted in 1984). The biosynthesis, control and mode of action of ethylene in Penicillium digitatum-infected citrus fruit. Ph.D. thesis, to be submitted to the Faculty of Agriculture of the Hebrew University of Jerusalem, Israel.

List of publications and abstracts

1. Chalutz, E., A.K. Mattoo and Y. Fuchs (1980). Biosynthesis of ethylene: the effect of phosphate. *Plant, Cell and Environ.* 3:349-356.
2. Zauberman, G. and Y. Fuchs (1981). Effect of wounding on "Fuerte" avocado ripening. *HortScience* 16:496-497.
3. Fuchs, Y., A.K. Mattoo, E. Chalutz and Ilana Rot (1981). Biosynthesis of ethylene in higher plants. The metabolic site of inhibition by phosphate. *Plant, Cell and Environ.* 4:291-295.
4. Mattoo, A.K., O. Achilea, Y. Fuchs and E. Chalutz (1982). Membrane association and some characteristics of the ethylene forming enzyme from etiolated pea seedlings: *Biochem & Biophys. Res. Comm.* 195: 271-278.
5. Mattoo, A.K., D.O. Adams, G.W. Patterson and M. Lieberman (1982). Inhibition of 1-aminocyclopropane-1-carboxylic acid synthase by phenothiazines. *Plant Sci. Letters.* 28:173-179.
6. Anderson, J.D., A.K. Mattoo and M. Lieberman (1982). Induction of ethylene biosynthesis in tobacco leaf discs by cell wall digesting enzymes. *Bioch. and Biophys. Res. Comm.* 107:588-596.
7. Esther Kapulnik, A.K. Mattoo, E. Chalutz and I. Chet (1982). The relationship between the production of ethylene by certain fungi, their enzyme constitution and their ability to accumulate intracellular phosphate. *Z. Pflanzenphysiol.* 109:347-354.
8. Lisker, N., Lea Cohen, E. Chalutz and Y. Fuchs (1983). Fungal infections suppress ethylene-induced phenylalanine ammonia-lyase activity in grapefruits. *Physiol. Plant Pathol.* 22:331-338.
9. Mattoo, A.K., E. Chalutz, J.D. Anderson and M. Lieberman (1983). Variation in adenylates and adenylate energy charge during phosphate-mediated inhibition of ethylene biosynthesis in Penicillium digitatum. *Z. Pflanzenphysiol.* 111:301-309.
10. Chalutz, E., A.K. Mattoo, T. Solomos and J.D. Anderson (1984). Enhancement by ethylene of Cellulysin-induced ethylene production by tobacco leaf discs. *Plant Physiol.* 74: 99-103.

11. Achilea, O., E. Chalutz, Y. Fuchs and I. Rot (1984). Ethylene biosynthesis and related physiological changes in Penicillium digitatum-infected citrus fruit. *Physiol. Plant Pathol.* (Submitted).
12. Fuchs, Y., E. Chalutz, I. Rot and A.K. Mattoo (1980). Site of phosphate inhibition of ethylene biosynthesis in higher plants. *Plant Physiol.* 65 Suppl. p. 43, Abstr. No. 272.
13. Mattoo, A.K., Y. Fuchs and E. Chalutz (1981). Regulatory aspects of ethylene biosynthesis in higher plants and microorganisms. *Isr. J. Bot.* 30:55 (Abstr).
14. Chalutz, E., Y. Fuchs and A.K. Mattoo (1981). Ethylene production by some microorganisms: Role in pathogenicity and regulation by phosphate. *Isr. J. Bot.* 30:54 (Abstr).
15. Mattoo, A.K. and M. Lieberman (1982). Role of silver ions controlling senescence and conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene. *Plant Physiol.* 69 Suppl. p. 18, Abstr. No. 94.
16. Anderson, J.D., A.K. Mattoo and M. Lieberman (1982). Cycloheximide-insensitive induction of ethylene biosynthesis in tobacco leaf discs by cell wall digesting enzymes. *Proc. 11th International Confer. on Plant Growth Regulators* p. 12, Abstr. No. 305.
17. Mattoo, A.K., J.E. Baker and M. Lieberman (1983). Copper induced ethylene production by Spirodela oligorrhiza is a consequence of singlet oxygen mediated membrane damage. *Plant Physiol. Suppl.* 72, p. 39. Abstr. No. 215.
18. Achilea, O., E. Chalutz, Y. Fuchs and Ilana Rot (1983). Ethylene biosynthesis and related physiological changes in Penicillium digitatum-infected citrus fruit. *Plant Physiol. Suppl.* 72, p. 163, Abstr. No.925.
19. Chalutz, E., A.K. Mattoo, T. Solomos and J.D. Anderson (1983). Enhancement by ethylene of Cellulysin-induced ethylene production by tobacco leaf discs. *Plant Physiol. Suppl.* 72 p.158, Abstr. No.902.
20. Chalutz, E., A.K. Mattoo and J.D. Anderson (1983). The relationship between cellulysin-induced ethylene production by tobacco leaf discs and ethylene produced during host-pathogen interaction. *Proceedings of the 10th Annual Meeting of the Plant Growth Regulator Society of America*, 18-24.

21. Anderson, J.D., E. Chalutz and A.K. Mattoo (1984). Purification and properties of ethylene inducing factor from the cell wall digesting enzyme mixture, cellulysin. International Conference on Biochemical, Physiological and Applied Aspects of Ethylene, Jerusalem, Israel, January 1984 (Abstract).
22. Mattoo, A.K. and J.D. Anderson (1984). Characterization of wound-induced 1-aminocyclopropane-1-carboxylic acid synthase activity. International Conference on Biochemical, Physiological and Applied Aspects of Ethylene. Jerusalem, Israel, January 1984. (Abstract).
23. Chalutz, E., A.K. Mattoo and J.D. Anderson (1984). Enhancement by ethylene of cellulysin-induced ethylene production by tobacco leaf discs. International Conference on Biochemical, Physiological and Applied Aspects. Jerusalem, Israel, January 1984 (Abstract).
24. Achilea, O., E. Chalutz, Y. Fuchs and I. Rot. (1984). Ethylene biosynthesis in Penicillium digitatum-infected citrus fruit. 39th Easter School Conference on Ethylene and Plant Development. Nottingham, England. (Abstr).

Table 1 The influence of the pH of the water extract of the peel on the solubility of its proteins.

Peel Tissue	Water soluble proteins in the extract		
	Non-treated (mg g ⁻¹)	Modified to pH 4.5 (% of non-treated)	Modified to pH 3.5 (% of non-treated)
Albedo	1.87 ± 0.062	82.4 ± 6.6	80.0 ± 4.6
Flavedo	3.15 ± 0.073	80.9 ± 3.9	75.9 ± 5.9

Water extracts were prepared from peel tissues of healthy grapefruits. The pH of the extracts was either non-treated or modified (by HCl) to 4.5 or 3.5. The extracts were then incubated at 0°C for 8 h and water-soluble proteins were determined. The original pH of the extracts of the albedo and the flavedo were 5.2 ± 0.05 and 5.4 ± 0.06, respectively. Each value is a mean of 9 replicates with S.D.

Table 2 ACC content and conversion of ACC to ethylene by albedo discs, cut from different locations of P. digitatum - infected grapefruit.

Assayed site	Control		ACC treated
	ACC content (nmol g ⁻¹)	Ethylene production (nl g ⁻¹ h ⁻¹)	Ethylene production (% of control)
+70	0.5 ± 0.03	0.2 ± 0.06	994 ± 130
+8	8.8 ± 2.7	2.3 ± 0.8	142 ± 31
-8	18.1 ± 3.0	0.2 ± 0.08	102 ± 24
-70	32.4 ± 4.2	2.0 ± 0.6	91 ± 17

Albedo discs were excised from the indicated sites (see Fig. 1A) of P. digitatum - infected grapefruit. Five discs of each site were treated with either water (control), or with ACC (5mM) and incubated at 24°C for 6 h in the dark. ACC content and ethylene production were then determined. ACC content of the ACC-treated discs ranged from 141 to 180 nmol g⁻¹. Each value in the table is a mean of 5 replicates with S.D.

Figure 1 Some biochemical and physiological changes in the peel of P. digitatum-infected grapefruit in relation to ACC content and ethylene production.

A: Definitions of regions on a 6-days old infected grapefruit and the method of their graphic presentation as a function of their distance from the maceration front. B to G: Changes in biochemical and physiological parameters, in an infected fruit as related to the distance from the maceration front. B: Glucosamine content. C: Galacturonic acid. D: pH of water extract. E: Water-soluble protein content in water extract. F: ACC content. G: Ethylene production.

All assays were carried out on peel tissues samples taken from healthy or P. digitatum-infected grapefruits, 6 days after ^oinculation near the stem-end of the fruit. The fruits were incubated at 21°C in the dark. Assay procedures are given in "Materials and Methods". Bars indicate S.D.

○————○:Healthy fruit albedo. Δ-----Δ:Healthy fruit flavedo.
 ●————●:Infected fruit albedo. ▲-----▲:Infected fruit flavedo.

Figure 2 Time-course of ethylene production by albedo and flavedo discs, cut from different locations of healthy or P. digitatum-infected grapefruit.

Ethylene production by peel discs cut from different sites of the fruit, as defined in Fig. 1A: +70(A); +8(B); -8(C); and -70(D).

○————○: Healthy fruit albedo. Δ-----Δ: Healthy fruit flavedo.
 ●————●: Infected fruit albedo. ▲-----▲: Infected fruit flavedo.

Five peel discs, 12 mm in diameter were excised from each site of nine fruits. The discs were separated into the albedo and flavedo parts, weighed and placed inside a 21 ml glass vial. The vials were incubated

in the dark at 24⁰C. One h accumulations of ethylene were determined at various intervals during the following 32 h. Prior to every closure, the vials were aerated. The data obtained from healthy control discs are not given for Fig. 2B,C and D, since they were similar to those shown in Fig. 2A. Vertical bars indicate S.D.

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Figure 1