ETHYLENE AND PLANT DEVELOPMENT

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PREFACE

This volume contains the Proceedings of the Thirty-ninth University of Nottingham Easter School in Agricultural Science which was held at Sutton Bonington from 26th–30th March 1984. The conference was entitled 'Ethylene and Plant Development' and included a workshop, organized in conjunction with the Association of Applied Biologists, on the 'Practical control of ethylene in fruit, vegetables and flowers'. The contents are a mixture of review and research papers thus giving a thorough and up-to-date presentation of the subject. Ethylene is of great agricultural and horticultural significance by virtue of its role in such developmental processes as growth, ripening, abscission and senescence. The workshop reviewed the practical methods and advantages of either applying ethylene to, or removing ethylene from, various commercial products. The rest of the conference dealt with the more fundamental aspects of ethylene synthesis and action during the developmental processes in which the gas is active. Emphasis was particularly placed on the effects of ethylene on gene expression and cell development since advances in these areas may eventually lead to a more scientifically-based control of ethylene levels and action within the plant. The organizers gratefully acknowledge the financial support of ICI, Bayer AG, Monsanto and Shell. The success of the conference was largely due to the administrative skills and patience of Mrs E. Wyss and Mrs S. Bruce.

Thanks also go to Dr M. Knee for his assistance in the organization of the workshop, and to Dr F.B. Abeles, Professor S.F. Yang, Professor J. Bruinsma, Dr M.B. Jackson, Dr R.O. Sharpes and Dr D.J. Osborne for their confident chairing of the various sessions. Finally we would like to thank all the participants, both delegates and helpers, for contributing to such an enjoyable conference.

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ETHYLENE AND PLANT DEVELOPMENT: AN INTRODUCTION

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Introduction

Early work on ethylene dealt with reports on the effects of leaking illuminating gas on plants (Girardin, 1864). In 1901, Neljubow (1901) demonstrated that ethylene was the physiologically active ingredient of illuminating gas. Later, Crocker and Knight (1908) reported that ethylene was a potent plant growth regulator and that it was capable of causing floral senescence, epinasty, abscission, intumescences and inhibition of growth. They also noted that as little as $0.1\,\mu l\, l^{-1}$ was capable of causing these various effects. The ability of gases produced by oranges to ripen bananas was noted by Cousins (1910), and later Denny (1924) reported that ethylene accelerated the ripening and respiration of lemons. The work by Kidd and West (1925) on the climacteric was seminal in focusing attention on the varied metabolic changes in fruit ripening and set the stage for contemporary research on fruit storage physiology.

Research on phytohormones was accelerated with the discovery of auxin in 1925 (Went and Thimann, 1937). However, the greater appeal of working with indole-acetic acid (IAA), which was known to be produced by plants as opposed to ethylene whose presence was difficult to quantitatively identify, captured the energies and attention of most plant hormone physiologists. Gane (1934) provided chemical evidence for the production of ethylene by plants, but it was not until Burg and Stolwijk (1959) showed that gas chromatography could be used to quantitatively measure physiologically significant levels of ethylene that the field attracted significant numbers of workers.

The concept that ethylene might be an important second messenger in plant development was initiated with the observation by Zimmerman and Wilcoxon (1935) that auxin increased ethylene production and that the ethylene so produced might play a role in auxin action. They suggested that auxin-induced ethylene production might play a role in the ability of auxin to induce epinasty, swelling, root initiation and inhibition of growth. We now know that auxin-induced ethylene production plays a role in many processes (*Table 1.1*).

As mentioned above, ethylene enhances fruit ripening. Regeimbal and Harvey (1927) reported that both invertase and protease activity of pineapples increased after they were treated with ethylene suggesting that the control of enzyme synthesis might play a role in ethylene action. Since that time, ethylene has been

Table 1.1 DEVELOPMENTAL PROCESSES WHERE AUXIN-INDUCED ETHYLENE PRODUCTION IS THOUGHT TO MEDIATE AUXIN ACTION

Abscission (Abeles and Rubinstein, 1964)
Apical dominance (Blake, Reid and Rood, 1983)
Branch angle (Blake, Pharis and Reid, 1980)
Bud growth, inhibition (Burg and Burg, 1968)
Callus, shoot initiation and growth (Huxter, Thorpe and Reid, 1981)
Epinasty (Amrhein and Schneebeck, 1980)
Flowering inhibition (Abeles, 1967)
Flowering, promotion in bromeliads (Burg and Burg, 1966b)
Flowering, senescence (Burg and Dijkman, 1967)
Flowering, sex expression in cucurbits (Shannon and de le Guardia, 1969)
Hypertrophy of hypocotyls (Wample and Reid, 1979)
Hypocotyl hook opening (Kang *et al.*, 1967)
Isocoumarin formation in carrots (Chalutz, De Vay and Maxie, 1969)
Latex flow, promotion (D'Auzac and Ribaillier, 1969)
Phenylalanine ammonia lyase (Rhodes and Wooltorton, 1971)
Root elongation, inhibition (Chadwick and Burg, 1970)
Root initiation (Fabijan, Taylor and Reid, 1981)
Stem elongation, inhibition (Burg and Burg, 1966a)
Swelling, onion leaf bases (Levy and Kedar, 1970)

Table 1.2 ENZYMES REGULATED BY ETHYLENE

Abscission
  Cellulase (Horton and Osborne, 1967)
  Polygalacturonase (Hashinaga *et al.*, 1981)
Aerenchyma
  Cellulase (Kawase, 1981)
Ripening
  Cellulase (Pesis, Fuchs and Zauberman, 1978)
  Chlorophyllase (Looney and Patterson, 1967)
  Invertase (Jeffery *et al.*, 1984)
  Laccase (Mayer and Harel, 1981)
  Malate dehydrogenase (Rhodes *et al.*, 1968)
  Polygalacturonase (Grierson, Tucker and Robertson, 1981)
Senescence
  Ribonuclease (Sacher, Engstrom and Broomfield, 1979)
Stress
  Beta-1,3-glucanase (Abeles *et al.*, 1971)
  Chitinase (Boller *et al.*, 1983)
  Cinnamate 4-hydroxylase (Rhodes, Wooltorton and Hill, 1981)
  Hydroxycinnamate CoA ligase (Rhodes, Wooltorton and Hill, 1981)
  Hydroxyproline rich glycoprotein (Toppan, Roby and Esquerre-Tugaye, 1982)
  Phenylalanine ammonia lyase (Rhodes, Wooltorton and Hill, 1981)
Function not known
  Ethylene mono-oxygenase (Abeles and Dunn, 1984)
  Peroxidase (Gahagan, Holm and Abeles, 1968)

Table 1.3 INHIBITORS OF PYRIDOXAL PHOSPHATE DEPENDENT ENZYMES

Rhizobitoxin: alpha-amino-gamma-(2′-amino-3′-hydroxypropoxy)-trans-beta-butenoic acid.
  $\mathrm{HOCH_2\text{-}CHNH_2\text{-}CH\text{-}O\text{-}CH{=}CH\text{-}CHNH_2\text{-}COOH}$
L-Canaline: alpha-amino-gamma-amino-oxybutyric acid
  $\mathrm{H_2N\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CHNH_2\text{-}COOH}$
AVG: L-alpha-amino-gamma-(2′amino-ethoxy)-trans-beta-butenoic acid
  $\mathrm{H_2N\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH{=}CH\text{-}CHNH_2\text{-}COOH}$
AOA: Amino-oxyacetic acid
  $\mathrm{H_2N\text{-}O\text{-}CH_2\text{-}COOH}$

shown to increase the activity of a number of enzymes associated with ripening, abscission, senescence and stress. A partial list of the enzymes associated with these processes is given in *Table 1.2*.

Lieberman and Mapson (1964) were the first to show that methionine was a precursor of ethylene. Later, they demonstrated that rhizobitoxin was an effective inhibitor of ethylene production (Owens, Lieberman and Kunishi, 1971). Since that time, other inhibitors of pyridoxal phosphate dependent enzymes such as canaline, aminoethoxyvinylglycine (AVG), and amino-oxyacetic acid (AOA) have been used to unravel the pathway from methionine to ethylene (Amrhein and Wenker, 1979). The structures of these inhibitors are shown in *Table 1.3*.

Ethylene biosynthesis and action

While some of the details of the ethylene pathway are still being determined, the general outline is that methionine is converted to S-adenosylmethionine (SAM), then to 1-aminocyclo-propane-1-carboxylic acid (ACC), and finally to ethylene (Adams and Yang, 1979). The ethylene forming enzyme (EFE) has been difficult to study because it may be localized for instance on the vacuolar membrane (Guy and Kende, 1984) and activity of this enzyme is lost when membranes are destroyed (*see* Kende, Acaster and Guy, Chapter 3).

Some progress is being made on the mechanism of ethylene action. Valuable tools in these studies are the observations that CO_2 (Burg and Burg, 1967), silver ions (Beyer, 1976), hypobaric atmospheres (Burg and Burg, 1965) and the chemical TH6241 (Thompson Hayward Chemical Co.) (1,5-methyl-4-ethoxycarbonylmethoxy-1,2,3-benzothiodiazole) (Parups, 1973; Daalen and Daams, 1970) can block ethylene action. Additional aids in such studies are the concepts that a similarity exists between ethylene effects in terms of dose response curves and the effect of hydrocarbon gas analogues (Burg and Burg, 1967).

As far as we know, all plant cells make ethylene all the time. Because of this, its ability to act as a regulator is dependent on one of the following mechanisms. The first mechanism involves a change in the sensitivity of the cell to the ethylene that is already there while the second involves a response caused by a change in the level of ethylene produced by the tissue. In the first case, the rate of ethylene production remains constant during the physiological process. An example of this would be abscission. During abscission, it appears as if the ability of ethylene to act depends on the amount of auxin (acting as a juvenility factor) that is in the tissue (Abeles and Rubinstein, 1964). An example of a process which is controlled by an increase in the rate of ethylene production is wound-induced protein synthesis. Tissue damage results in an increase in ethylene production which in turn stimulates the synthesis of enzymes such as beta-1,3-glucanase and chitinase (Abeles *et al.*, 1971).

For ethylene to act it must bind to some part of the cell. As far as we know, one binding site seems to be used for most, if not all, ethylene effects. This interpretation is based on the results obtained from studies with ethylene action inhibitors, dose response curves and ethylene analogues. Though exceptions exist, many physiological effects of ethylene that are blocked with CO_2 and silver ions show similar dose response curves and respond similarly to various hydrocarbon analogues such as propylene and acetylene.

The problem of hormone binding, and the initial effects of the hormone binding site complex, has been a major research challenge. Ethylene has a special appeal for these studies because it is a simple molecule. It is also relatively easy to add and monitor ethylene in an experimental system. At the present time, investigators are evaluating the possibility that ethylene undergoes chemical modification at its site of action (*see* Beyer, 1981, and Chapter 12 for a review on ethylene action and metabolism). The binding (covalent, coordinate, or van der Waal's) between ethylene and its site of action can be reversible and relatively rapid. For example, the response time for epinasty (Funke *et al.*, 1938) and the inhibition of root elongation (Chadwick and Burg, 1970) is 1 h. In the case of ethylene induced inhibition of elongation of seedlings, about 30 min are required for ethylene action (Eisinger, 1983). In the case of epinasty, recovery from the effect of ethylene requires some hours, while in the root elongation system, removal of ethylene results in a rapid, almost instantaneous resumption of normal growth (Chadwick and Burg, 1970). In a similar fashion, Biale and Young (1981) indicated that ethylene caused a rapid and reversible increase in the rate of lemon fruit respiration.

Direct and indirect approaches have been used in hormone action studies. The direct approach has been to examine the physical binding of ethylene to the plant or plant parts. The indirect approach has been to study ethylene mediated processes such as ripening and work backwards, learning more about the details of earlier elements in the system. For example, ripening is a softening process involving the induction of polygalacturonase. The increase in polygalacturonase is preceded by the synthesis of its mRNA (Grierson, Tucker and Robertson, 1981 and Chapter 14). The same may also be true of abscission, another cell wall degrading process, involving cellulase (Abeles and Holm, 1966). The logical conclusion then is the belief that ethylene can activate a particular part of the nucleus. For these processes and others like them involving protein synthesis, ethylene alone or in conjunction with another substance, activates the genome.

The reports that ethylene promotes seed germination (Ketring, 1977; Taylorson, 1979) and bud break (Morgan, Meyer and Merkle, 1969) suggest that ethylene can also act by regulating the translation of preformed mRNA. Others have shown that such preformed or stored mRNA plays an important role in seed germination (Payne, 1976; Suzuki and Minamikawa, 1983). Since leaves may also contain stored mRNA (Giles, Grierson and Smith, 1977), processes involving the rapid production and response to ethylene such as wounding and stress may also involve stored mRNA.

The direct approach for ethylene action studies has been to look for binding sites by using labelled ethylene (Sisler, 1979, 1980). This approach faces a number of obstacles. The amount of ethylene needed for a physiological effect is small (about $0.1\ \mu l\ l^{-1}$, or 10^{-9} M in the liquid phase) so even with highly labelled ethylene, only small amounts are bound. The binding forces are weak. For example, as discussed above, the removal of ethylene from the gas phase surrounding stem or root tissue can result in a rapid return to normal growth rates. Finally, some plants have the ability to oxidize ethylene to ethylene oxide, carbon dioxide and other derivatives (Beyer, 1981), and the presence of these metabolic products complicates the interpretation of data.

We have used a modification of affinity chromatography to estimate the binding of ethylene to plants. In this technique, a pulse of ethylene or other hydrocarbon gas and methane acting as an internal standard are flushed through a glass column filled with ethylene sensitive tissue such as germinating seeds. The length of time required for the gases to appear in the effluent of this 'plant chromatograph' was used as an indication of their relative affinity for the tissue. For example, if the transit time of ethylene through the column was greater than that for methane, this would indicate binding. However, we have observed that the relative affinity of ethylene and other hydrocarbon gases for plant and fungal tissue was more closely associated with their solubility in water than any physiological activity. In addition, competitive inhibitors of ethylene action such as silver ions and CO_2 did not decrease binding (Abeles, 1984a).

The concept that ethylene acts without undergoing any dissociation has been tested a number of times. Earlier studies indicated that ethylene itself was not rearranged as a result of its contact with the cell (Beyer, 1981). While some early reports suggested that plants metabolize ethylene, it wasn't until the work of Jerie and Hall (1978), Dodds *et al.* (1979) and Beyer (1981) that ethylene oxidation by plants was conclusively demonstrated. The significance of this phenomenon is not fully understood and various explanations have been advanced. It is conceivable that oxidation of ethylene is a side reaction or effect of the ethylene binding site complex. For example, an increase in ethylene oxidation is associated with ripening, abscission and floral senescence (Beyer, 1981). Carbon disulphide (CS_2) has been a useful probe to test the role of ethylene oxidation in ethylene action. As Beyer originally observed, CS_2 was an effective inhibitor of ethylene oxidation. The action appears to be specific because CS_2 at the levels used in our experiments had no effect on growth, respiration and photosynthesis (Abeles, 1984b). We have observed that CS_2 totally blocked ethylene oxidation without inhibiting ethylene action. The reports cited above, that ethylene oxidation increases during ripening, abscission and floral senescence, suggested that ethylene may control the enzyme which oxidizes it. We have obtained evidence in favour of that view and have observed that ethylene can cause a manifold increase in oxidase activity and that the effect is blocked by cycloheximide (Abeles and Dunn, 1984).

Conclusion

We have learned much about ethylene and plant biology in the 83 years since a Russian graduate student showed that the ethylene in illuminating gas caused pea seedlings to grow horizontally (Neljubow, 1901). Through the efforts of many workers we currently possess a good deal of information on what ethylene does and how the cell synthesizes it. We have also developed a finer appreciation of the normal role of ethylene in plant growth and development. The need to learn more about the initial binding sites and the effects of the bound ethylene still remains a major challenge. Even though this goal remains elusive, we are learning to ask better questions, use better tools, and design more sophisticated experiments.

Agriculture has been quick to exploit the lessons learned thus far in this field of phytohormones. For example, most fruit and vegetable storage strategies include attempts to remove or eliminate ethylene action. Ethrel (2-chloroethyphosphonic acid), an ethylene releasing compound, is being incorporated in a variety of horticultural practices. It is safe to assume that the work presented here, and to be performed in the future, will also benefit agriculture and basic plant biology.

References

ABELES, F.B. (1967), *Plant Physiology*, **42**, 608–609
ABELES, F.B. (1984a). *Plant Physiology*, **74**, 525–528
ABELES, F.B. (1984b). *Journal of Plant Growth Regulation*, **3**, 85–89

ABELES, F.B., BOSSHART, R.P., FORRENCE, L.E., and HABIG, W.H. (1971). *Plant Physiology*, **47**, 129–134

ABELES, F.B. and DUNN, L.S. (1984). *Plant Physiology* (submitted for publication)

ABELES, F.B. and HOLM, R.E. (1966). *Plant Physiology*, **41**, 1337–1342

ABELES, F.B. and RUBINSTEIN, B. (1964). *Plant Physiology*, **39**, 963–969

ADAMS, D.O. and YANG, S.F. (1979). *Proceedings of the National Academy of Sciences*, **76**, 170–174

AMRHEIN, N. and SCHNEEBECK, D. (1980). *Physiologia Plantarum*, **49**, 62–64

AMRHEIN, N. and WENKER, D. (1979). *Plant and Cell Physiology*, **20**, 1635–1642

BEYER, E.M. (1976). *Plant Physiology*, **58**, 268–271

BEYER, E.M. (1981). In *Recent Advances in the Biochemistry of Fruits and Vegetables*, pp. 107–121. Ed. by Friend, J. and Rhodes, M.J.C. Phytochemical Society of Europe. Symposium Series No. 19. Academic Press, New York

BIALE, J.B. and YOUNG, R.E. (1981). In *Recent Advances in the Biochemistry of Fruits and Vegetables*, pp. 1–9. Ed. by Friend, J. and Rhodes, M.J.C. Phytochemical Society of Europe. Symposium Series No. 19. Academic Press, New York

BLAKE, T.J., PHARIS, R.P. and REID, D.M. (1980). *Planta*, **148**, 64–68

BLAKE, T.J., REID, D.M. and ROOD, S.B. (1983). *Physiologia Plantarum*, **59**, 481–487

BOLLER, T., GEHRI, A., MAUCH, F. and VÖGELI, U. (1983). *Planta*, **157**, 22–31

BURG, S.P. and BURG, E.A. (1965). *Science*, **148**, 1190–1196

BURG, S.P. and BURG, E.A. (1966a). *Proceedings of the National Academy of Sciences*, **55**, 262–269

BURG, S.P. and BURG, E.A. (1966b). *Science*, **152**, 1269

BURG, S.P. and BURG, E.A. (1967). *Plant Physiology*, **42**, 144–152

BURG, S.P. and BURG, E.A. (1968). *Plant Physiology*, **43**, 1069–1074

BURG, S.P. and DIJKMAN, M.J. (1967). *Plant Physiology*, **42**, 1648–1650

BURG, S.P. and STOLWIJK, J.A.J. (1959). *Journal of Biochemistry and Microbiological Technology and Engineering*, **1**, 245–259

CHADWICK, A.V. and BURG, S.P. (1970). *Plant Physiology*, **45**, 192–200

CHALUTZ, E., DE VAY, J.E. and MAXIE, E.C. (1969). *Plant Physiology*, **44**, 235–241

CROCKER, W. and KNIGHT, L.I. (1908). *Botanical Gazette*, **46**, 259–276

COUSINS, H.H. (1910). *Annual Report of the Department of Agriculture, Jamaica*

DAALEN, J.J. VAN and DAAMS, J. (1970). *Naturwissenschaften*, **8**, 395

D'AUZAC, J. and RIBAILLIER, D. (1969). *Comptes Rendus de L'Academie des Sciences, Paris*, **268**, 3046–3050

DENNY, F.E. (1924). *Journal of Agricultural Research*, **27**, 757–769

DODDS, J.H., MUSA, S.K., JERIE, P.H. and HALL, M.A. (1979). *Plant Science Letters*, **17**, 109–114

EISINGER, W. (1983). *Annual Reviews of Plant Physiology*, **34**, 225–240

FABIJAN, D., TAYLOR, J.S. and REID, D.M. (1981). *Physiologia Plantarum*, **53**, 589–597

FUNKE, G.L., DE COEYER, F., DE DECKER, A. and MATON, J. (1938). *Biologisch Jaarboek*, **5**, 335–381

GAHAGAN, H.E., HOLM, R.E. and ABELES, F.B. (1968). *Physiologia Plantarum*, **21**, 1270–1279

GANE, R. (1934). *Nature (London)*, **134**, 1008

GILES, A.B., GRIERSON, D. and SMITH, H. (1977). *Planta*, **136**, 31–36

GIRARDIN, J.P.L. (1864). *Jahrerbauch über die Agrikulture-Chemie*, **7**, 199–200

GRIERSON, D., TUCKER, G.A. and ROBERTSON, N.G. (1981). In *Recent Advances in* *the Biochemistry of Fruits and Vegetables*, pp. 149–160. Ed. by Friend, J. and Rhodes, M.J.C. Phytochemical Society of Europe. Symposium Series No. 19. Academic Press, New York

GUY, M. and KENDE, H. (1984). *Planta*, **160**, 281–287

HASHINAGA, F., IWAHORI, S., NISHI, Y. and ITOO, S. (1981). *Nippon Nogeikagaku Kaishi*, **55**, 1217–1223

HORTON, R.F. and OSBORNE, D.J. (1967). *Nature (London)*, **214**, 1086–1088

HUXTER, T.J., THORPE, T.A. and REID, D.M. (1981). *Physiologia Plantarum*, **53**, 319–326

JEFFERY, D., SMITH, C., GOODENOUGH, P., PROSSER, I. and GRIERSON, D. (1984). *Plant Physiology*, **74**, 32–38

JERIE, P.H. and HALL, M.A. (1978). *Proceedings of the Royal Society of London B*, **200**, 87–94

KANG, B.G., YOCUM, C.S., BURG, S.P. and RAY, P.M. (1967). *Science*, **156**, 958–959

KAWASE, M. (1981). *American Journal of Botany*, **68**, 651–658

KETRING, D.L. (1977). In *Physiology and Biochemistry of Seed Dormancy and Germination*, pp. 136–178. North Holland Publ. Co., Amsterdam

KIDD, F. and WEST, C. (1925). *Great Britain Department of Science and Industry Research. Food Investigation Board Report. 1924*, 27–33

LEVY, D. and KEDAR, N. (1970). *HortScience*, **5**, 80–82

LIEBERMAN, M. and MAPSON, L.W. (1964). *Nature (London)*, **204**, 343–345

LOONEY, N.E. and PATTERSON, M.E. (1967). *Nature (London)*, **214**, 1245–1246

MAYER, A.M. and HAREL, E. (1981). In *Recent Advances in the Biochemistry of Fruits and Vegetables*, pp. 161–180. Ed. by Friend, J. and Rhodes, M.J.C. Phytochemical Society of Europe. Symposium Series No. 19. Academic Press, New York

MORGAN, P.W., MEYER, R.E. and MERKLE, M.G. (1969). *Weed Science*, **17**, 353–355

NELJUBOW, D. (1901). *Beihefte zur Botanisches Centralblatt*, **10**, 128–129

OWENS, L.D., LIEBERMAN, M. and KUNISHI, A. (1971). *Plant Physiology*, **48**, 1–4

PARUPS, E.V. (1973). *Physiologia Plantarum*, **29**, 365–370

PAYNE, P.I. (1976). *Biological Review*, **51**, 329–363

PESIS, E., FUCHS, Y. and ZAUBERMAN, G. (1978). *Plant Physiology*, **61**, 416–419

REGEIMBAL, L.O. and HARVEY, R.B. (1927). *Journal of the American Chemical Society*, **49**, 1117–1118

RHODES, M.J.C., GALLIARD, T., WOOLTORTON, L.S.C. and HULME, A.C. (1968). *Phytochemistry*, **7**, 405–408

RHODES, M.J.C. and WOOLTORTON, L.S.C. (1971). *Phytochemistry*, **10**, 1989–1997

RHODES, M.J.C., WOOLTORTON, L.S.C. and HILL, A.C. (1981). In *Recent Advances in the Biochemistry of Fruits and Vegetables*, pp. 191–218. Ed. by Friend, J. and Rhodes, M.J.C. Phytochemical Society of Europe. Symposium Series No. 19. Academic Press, New York

SACHER, J.A., ENGSTROM, D. and BROOMFIELD, D. (1979). *Planta*, **144**, 413–418

SHANNON, S. and DE LE GUARDIA, M.D. (1969). *Nature (London)*, **223**, 186

SISLER, E.C. (1979). *Plant Physiology*, **64**, 538–542

SISLER, E.C. (1980). *Plant Physiology*, **66**, 404–406

SUZUKI, Y. and MINAMIKAWA, T. (1983). *Plant and Cell Physiology*, **24**, 1371–1377

TAYLORSON, R.B. (1979). *Weed Science*, **27**, 7–10

TOPPAN, A., ROBY, D. and ESQUERRE-TUGAYE, M-T. (1982). *Plant Physiology*, **70**, 82–86

WAMPLE, R.L. and REID, D.M. (1979). *Physiologia Plantarum*, **45**, 219–226

WENT, F.W. and THIMANN, K.V. (1937). *Phytohormones*. Macmillan, New York
ZIMMERMAN, P.W. and WILCOXON, F. (1935). *Contributions of the Boyce Thompson Institute*, **7**, 209–229

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METABOLISM OF 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID

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Introduction

ACC (1-aminocyclopropane-1-carboxylic acid) was first isolated over 25 years ago from ripe cider apples and perry pears by Burroughs (1957) and from ripe cowberries by Vahatalo and Virtanen (1957). Although Burroughs (1960) could not detect ACC in other varieties of apple or pear he examined, he observed that the amount of ACC in perry pears increased during storage, and speculated that ACC might be related in some way to fruit ripening. However, ACC remained simply as one of the non-protein amino acids of plants, arousing little interest until its recognition as an ethylene precursor in 1979. Since Adams and Yang (1979) demonstrated that ethylene is biosynthesized in apple tissue via the following sequence: methionine → S-adenosylmethionine (SAM) → ACC → ethylene, this pathway has been shown to operate throughout the diversity of higher plant tissues (Yang and Hoffman, 1984). The formation of ACC from SAM is catalysed by ACC synthase, which is considered to control the rate-limiting step in ethylene biosynthesis. In addition to the metabolism of ACC to ethylene, ACC has recently been recognized to be conjugated into N-malonyl-ACC (MACC).

In this chapter we shall summarize the present status of knowledge pertaining to the metabolism of ACC to ethylene and to MACC in various plant systems.

Metabolism of ACC to ethylene

REGULATION OF THE ETHYLENE-FORMING ENZYME (EFE) SYSTEM *IN VIVO*

When ACC is applied to various plant organs (with the exception of preclimacteric fruit and flowers) from a number of plant species, a marked increase in ethylene production is observed (Cameron *et al.*, 1979; Lürssen, Naumann and Schröder, 1979). This suggests that the enzyme system which converts ACC to ethylene (EFE) is largely constitutive and the formation of ACC is the rate-limiting step in these plant tissues. In preclimacteric fruit and young petals of carnation flower, ethylene production is very low because they have a very limited ability not only to convert SAM to ACC, but also to convert ACC to ethylene. However, at the onset of ripening or senescence, their ability to convert SAM to ACC and ACC to ethylene increases dramatically resulting in a surge in ethylene production. Thus, EFE can be induced during certain developmental stages. Since preclimacteric (unripe) fruit lack both ACC synthase and EFE, a massive increase in ethylene production requires development of both enzymes. However, when green tomato fruit were treated with ethylene for a short period (18 h), there was no increase in ACC content or in ethylene production rate, however the tissue's ability to convert ACC to ethylene increased markedly (*Table 2.1*). These data indicate that when preclimacteric fruit tissues are exposed to ethylene, the increase in EFE precedes the increase in ACC synthase. Whether or not this is also true during the natural ripening of fruits remains to be clarified. Ethylene is also known to promote the development of EFE in other excised tissues (Hoffman and Yang, 1982; Riov and Yang, 1982; Chalutz *et al.*, 1984). EFE can also be promoted by various environmental factors such as water stress (McKeon, Hoffman and Yang, 1982).

Table 2.1 PROMOTION BY ETHYLENE OF THE CAPABILITIES FOR CONVERTING ACC TO ETHYLENE AND MACC IN PRECLIMACTERIC TOMATO FRUIT

Pretreatment	*C_2H_4 production* (nmol g^{-1})	*ACC levels* (nmol g^{-1})	*ACC$\rightarrow C_2H_4$ production* (nmol g^{-1})	*ACC$\rightarrow$MACC levels* (nmol g^{-1})
Air	1.4	1.6	8.7	2.2
Ethylene	1.3	0.4	48.4	25.6

Immature green tomato fruits were treated with air or 10 μl l^{-1} ethylene for 18 h. Discs (0.5 cm diameter) were then prepared from the pericarp tissue, and ethylene produced and ACC content at the end of a 6 h incubation period were determined. For measurement of the capabilities to convert ACC to ethylene or to MACC, the discs were incubated in a solution containing 2 mM ACC and the amount of ethylene produced during, and MACC accumulated at the end of, a 6 h incubation period were determined

Recently many investigators have observed that light markedly inhibited ethylene production by various green leaf tissues in enclosed systems. Gepstein and Thimann (1980) were the first to report that the conversion of ACC to ethylene was inhibited by light. Since CO_2 is known to promote ethylene production in leaf tissues (Dhawan, Bassi and Spencer, 1981), Grodzinski, Boesel and Horton (1982) and Kao and Yang (1982) reasoned that the inhibition of ethylene evolution by light might result from a decrease in internal CO_2 concentration. Indeed, when CO_2 was added into the incubation flask, the rate of ethylene production in the light increased markedly, to a level which was even higher than that produced in the dark; carbon dioxide, however, had no appreciable effect on leaf segments incubated in the dark. The concentration of CO_2 giving half-maximal activity was 0.06% and 0.18% for rice and tobacco leaves, respectively. Thus, it is the CO_2 metabolism rather than light *per se*, which regulates the conversion of ACC to ethylene. Unlike the development of EFE mentioned above, the modulation of ACC conversion to ethylene by CO_2 or light is rapid and reversible, indicating that CO_2 regulates the activity, but not the synthesis of EFE (Kao and Yang, 1982). The mechanism by which CO_2 modulates the conversion of ACC to ethylene is not understood.

SOME CHARACTERISTICS OF EFE

It has long been recognized that dinitrophenol, high temperature, various lipophilic compounds, and osmotic shock treatment, all of which could modify membrane structure and function, greatly reduce the rate of ethylene synthesis in plant tissues. Moreover, when those tissues which are producing ethylene actively are homogenized, the ethylene-forming capability is totally lost. These observations lead to the suggestion that the ethylene-forming system is highly structured and requires membrane integrity (Lieberman, 1979). Recently, John (1983) has suggested that the generation of ethylene from ACC is coupled to a transmembrane flow of protons from the outside to the inside of the plasma membrane. This model explains the strict dependence of ethylene biosynthesis on membrane integrity and the marked inhibition by the protonophore, dinitrophenol. While this is an interesting hypothesis, direct experimental evidence is lacking.

Although the ACC molecule possesses two enantiotopic methylene groups, they are not geometrically equivalent and can be distinguished by a regiospecific enzyme. Ethyl substitution at one of each of the four methylene hydrogens results in four stereoisomers of 1-amino-2-ethylcyclopropane-1-carboxylic acid (AEC). If ACC conversion to ethylene by plant tissues were to proceed in regiospecific fashion, Hoffman *et al.* (1982a) reasoned that these four stereoisomers of AEC might not be converted into 1-butene with equal efficiency. In apple and etiolated mungbean hypocotyls, (+)-allocoronamic acid (for structure, *see Figure 2.2*) was preferentially converted to 1-butene. By chemical oxidation using NaOCl, in contrast, all AEC isomers were converted with nearly equal efficiency to 1-butene. ACC and AEC appear to be degraded by the same enzyme since both reactions are inhibited to the same extent by nitrogen atmosphere or by Co^{2+}, and since, when both substrates are present simultaneously, each acts as an inhibitor with respect to the other. These observations indicate that the enzyme converting ACC to ethylene exhibits regiospecificity.

Soon after ACC was established as the immediate precursor of ethylene, Konze and Kende (1979) reported an enzyme extract from etiolated pea seedling capable of converting ACC to ethylene. Many similar systems have since been reported, including carnation microsomes (Mayak, Legge and Thompson, 1981), pea microsomes (McRae, Baker and Thompson, 1982), pea mitochondria (Vinkler and Apelbaum, 1983), IAA-oxidase and peroxidase (Vioque, Albi and Vioque, 1981). Although these systems are oxygen-dependent, heat-denaturable and inhibited by radical scavengers, there are many characteristics which do not resemble those of the natural *in vivo* system. McKeon and Yang (1984) have compared the regiospecificity, with regard to the conversion of AEC isomers to 1-butene, by pea epicotyls and by the pea epicotyl extract. While pea epicotyls displayed the same regiospecificity observed in mungbean hypocotyls and apple tissues, and exhibited high affinity for ACC with a K_m of about 66 μM with respect to the internal ACC concentration, the pea homogenate did not differentiate between AEC isomers (*Table 2.2*) and exhibited very low affinity for ACC (reported Kms for the pea enzyme range from 15–400 mM). Moreover, the pea enzyme required Mn^{2+}, and was very sensitive to inhibition by EDTA and mercaptoethanol, whereas the *in vivo* system was not. These data contradict the view that the reported enzymic systems function to catalyse the conversion of ACC to ethylene *in vivo*. It should be noted that ACC can be converted to ethylene chemically by various oxidants, including oxidative free radicals (Legge, Thompson and Baker, 1982). A simple explanation accounting for the low affinity and lack of regiospecificity in these enzyme systems is that the physiological enzyme (EFE) catalyses directly the oxidation of ACC yielding ethylene, whereas the reported enzymes catalyse the activation of molecular oxygen probably to free radicals, such as O_2^- and OH, which