

USE OF ETHYLENE BIOSYNTHESIS INHIBITORS IN HORTICULTURE

Chien Yi Wang
Horticultural Science Institute
Agricultural Research Service
U.S. Department of Agriculture
Beltsville, Maryland 20705 USA

Summary

Inhibitors of ethylene biosynthesis, including aminoethoxyvinylglycine (AVG), aminoxyacetic acid (AOA), cobalt ion, free radical scavengers, low O₂ atmospheres, polyamines, and uncouplers of oxidative phosphorylation have been shown to cause various physiological effects in horticultural crops. The mechanism by which these inhibitors exert their effects is now better understood since the biosynthetic pathway of ethylene has been largely elucidated. These inhibitors have been used in a wide range of experiments. Their effects are reviewed and the implications of these findings for practical horticulture are discussed.

1. Introduction

Ethylene is beneficial in many aspects of horticultural practice (Sherman, 1985). However, ethylene can also be harmful at times. For example, it hastens the deterioration and shortens the life of most fruits, vegetables, and flowers. Inhibition of ethylene biosynthesis, therefore, reduces the detrimental effects that ethylene may cause.

Many compounds have been shown to inhibit ethylene biosynthesis. These compounds include AVG, AOA, cobalt ion, free radical scavengers, low O₂ atmospheres, polyamines, and uncouplers. Recent research has pinpointed the step in the ethylene biosynthetic pathway affected by each of these types of inhibitors (Yang and Hoffman, 1984). Figure 1 illustrates steps of inhibition by these inhibitors. Most of these inhibitors affect either conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) or conversion of ACC to ethylene. For example, the conversion of SAM to ACC, which is catalyzed by ACC synthase, is inhibited by AVG and AOA; whereas the conversion of ACC to ethylene, which is catalyzed by ethylene forming enzyme (EFE), is affected by cobalt ion, free radical scavengers, low O₂ atmospheres, polyamines, and uncouplers.

When the step between SAM and ACC is inhibited, endogenous ACC level is reduced and application of exogenous ACC can induce ethylene production. However, when the conversion of ACC to ethylene is inhibited, ACC accumulates and addition of exogenous ACC does not affect ethylene production.

2. Inhibition of ethylene biosynthesis by various compounds

2.1. Aminoethoxyvinylglycine (AVG)

AVG is an aminoethoxy analog of rhizobitoxine (Lieberman et al., 1974). Rhizobitoxine was first discovered in 1969 by Owens (1969) in the root nodules of a diseased soybean plant. It is a product of certain strains of the bacterium Rhizobium japonicum and was shown to be an inhibitor of β -cystathionase, an enzyme in the pathway of methionine biosynthesis in higher plants (Giovaneli et al., 1971). Subsequently, Owens et al. (1971) and Lieberman et al. (1974) found that rhizobitoxin and its analogs, particularly AVG, interfered with ethylene biosynthesis by blocking the conversion of methionine to ethylene and that this inhibition was irreversible. Following the elucidation of the biosynthetic pathway of ethylene in 1979, Adams and Yang (1979) demonstrated that the step of inhibition by AVG was between SAM and ACC.

2.2. Aminooxyacetic acid (AOA)

AOA is known to inhibit pyridoxal phosphate-mediated enzymatic reactions (John et al., 1979; Amrhein and Wenker, 1979). Yu et al. (1979a) reported that AOA was a potent inhibitor of ACC synthase. They also estimated that the K_i value for AOA was $0.8 \mu\text{M}$, as compared to a K_i value of $0.2 \mu\text{M}$ for AVG. Thus, ACC synthase is strongly inhibited by both AVG and AOA. AOA was shown to markedly inhibit IAA-induced ethylene production in mung bean hypocotyls and tomato fruit (Yu et al., 1979a, 1979b).

2.3. Cobalt ion (Co^{++})

Cobalt ion was shown to effectively inhibit ethylene production induced by IAA or kinetin in mung bean hypocotyl segments (Lau and Yang, 1976). Using tracer experiments, Yu and Yang (1979) identified that the step in ethylene biosynthetic pathway at which Co^{++} exerts its effect is between ACC and ethylene.

2.4. Free radical scavengers

Baker et al. (1978) showed that free radical scavengers, such as benzoate, propyl gallate, nordihydroguaiaretic acid, and eugenol inhibited ethylene production in vivo in green and pink tomato, apple, and avocado tissues. They proposed that free radical steps are universally involved in ethylene-forming systems. It was later suggested that free radicals mediate the step between ACC and ethylene (Apelbaum et al., 1981; Yang and Hoffman, 1984).

2.5. Low O_2 atmospheres

The requirement of oxygen for ethylene production in fruit was first observed in apples and pears by Gane (1934) and Hansen (1942). Burg and Thimann (1959) later suggested that lack of oxygen caused the accumulation of an ethylene precursor which could be converted to ethylene very rapidly upon exposure to air. In 1979, Adams and Yang (1979) examined the metabolism of methionine in the absence and presence of oxygen and identified this precursor as ACC. Low O_2 atmospheres have reduced ethylene production and extended storage life of many horticultural crops (Richardson and Meheriuk, 1982).

2.6. Polyamines

Exogenously applied polyamines have been shown to inhibit ethylene production in apple tissues (Apelbaum et al., 1981; Ben-Arie et al., 1982), soybean hypocotyls (Suttle, 1981), oat leaves (Fuhrer et al., 1982) and tobacco leaves (Apelbaum et al., 1981). The conversion of ACC to ethylene was reported to be inhibited rapidly by 1 to 10 mM of spermidine or spermine (Suttle, 1981; Apelbaum and Ickson, 1983). The inhibitory effect of polyamines on ethylene biosynthesis has been suggested to be due to their radical scavenging properties (Drolet et al., 1986).

2.7. Uncouplers

The uncouplers of oxidative phosphorylation, such as DNP have long been known to inhibit ethylene production in various plant tissues (Spencer, 1959; Lau et al., 1974; Murr and Yang, 1975). Previously it was thought that DNP only inhibited the step between methionine and SAM. However, recently it has been shown that the synthesis of ethylene from ACC is more vulnerable to DNP inhibition than the synthesis of SAM from methionine (Yu et al., 1980). Therefore, it was speculated that ATP may be required for the conversion of ACC to ethylene.

3. Responses of horticultural crops to inhibition of ethylene biosynthesis

3.1. Bloom delay

Dekazos (1979) applied AVG on intact branches of peach after completion of rest period and found that bud development was delayed. Preharvest application of AVG also delayed bloom of apple trees in the following year (Greene, 1983). Dennis et al. (1977) reported that AVG delayed the bloom not only in peach and apple but also in plum and sour cherry. The delay of bloom is beneficial in that the potential spring frost injury may be avoided.

3.2. Increase in vegetative growth

Application of AVG to apple trees in the fall has been found to increase vegetative growth in the following season. Increase in number of spurs and enhancement of spur elongation by AVG were shown on 'Red Delicious' apple trees (Williams, 1980; Greene, 1983). Increase in bud break and lateral branching has also been associated with AVG treatment (Williams, 1981; Curry and Williams, 1986). Ethylene production of apple lateral bud and apical shoot tip was reduced by AVG treatment (Williams, 1981; Curry and Williams, 1986). Increase in growth and number of spurs have an important practical implication. It means that younger trees may be able to produce a full crop and reach maturity faster if treated with an ethylene biosynthesis inhibitor.

3.3. Increase in fruit set and reduction of preharvest drop

When AVG was applied to 'Red Delicious' or 'Golden Delicious'

apple trees after full bloom, "June drop" was reduced and fruit set was increased (Williams, 1980). Improvement of fruit set on 'Richard Delicious' and 'Red Spur Delicious' by AVG sprays in the fall or at full-bloom has also been reported (Greene, 1983). The application of AVG within 1 month before harvest reduced preharvest drop and increased fruit removal force in trees of 'King of the Pippin' and 'Golden Delicious' apple (Bangerth, 1978). These effects are results of inhibition of ethylene biosynthesis. The seed number was increased and the number of parthenocarpic fruit was decreased by AVG treatment (Williams, 1981). This may also contribute to the increased retention of fruit.

3.4. Retention of fruit firmness and inhibition of fruit ripening

Preharvest application of AVG retarded softening and retained fruit firmness of 'Red Delicious' apples during storage (Williams, 1980). Delay of fruit ripening at room temperature was also observed in 'McIntosh', 'Spartan', 'Spencer', 'King of the Pippin', and 'Golden Delicious' apples after preharvest application of AVG (Bramlage et al., 1980; Bangerth, 1978).

Postharvest vacuum infiltration of AVG was also effective in inhibiting ripening of 'Anjou' and 'Bartlett' pears (Wang and Mellenthin, 1977; Ness and Romani, 1980). AVG treated fruits were still sensitive to ethylene application (Ness and Romani, 1980; Autio and Bramlage, 1982). Therefore, in a mixed storage, ethylene produced by untreated fruit will enhance ripening of treated fruit.

3.5. Delay of yellowing

Yellowing of some vegetables, such as broccoli, limits their marketing life. It was found that treatment with AVG and sodium benzoate, a free radical scavenger, reduced ethylene production, retarded the loss of chlorophyll and delayed yellowing of broccoli (Wang, 1977). Retardation of chlorophyll loss by cobalt ion and polyamines has also been shown in oat leaves (Gepstein and Thimann, 1981; Shih et al., 1982).

3.6. Induction of staminate flowers

Inhibition of ethylene biosynthesis by AVG leads to an enhancement of male tendency. Induction of staminate flowers on gynoecious cucumber and muskmelon by AVG has been reported (Atsmon and Tabbak, 1979; Loy et al., 1979; Owens et al., 1980).

3.7. Extending vase life of cut flowers

Most flowers are very sensitive to ethylene. The damaging effect of ethylene to the decorative quality of cut flowers is well known. It is conceivable that any treatment which decreases the ethylene production of flowers may delay senescence and prolong their vase life. AVG has been shown to extend the vase life of carnations, snapdragons, daffodils, irises, and chrysanthemums (Baker et al., 1977; Wang et al., 1977; Wang and Baker, 1979, 1980). In snapdragons, AVG also retarded the abscission of florets (Wang et al., 1977). Inhibition of ethylene production and extension of vase

life of carnations by AOA and free radical scavengers has also been demonstrated (Baker et al., 1977; Wang and Baker, 1980; Fujino et al., 1980; Broun and Mayak, 1981).

4. Concluding remarks

Ethylene biosynthesis inhibitors have been shown to have various effects on horticultural crops. In fruit trees, ethylene biosynthesis inhibitors were found to delay bloom, to increase vegetative growth, to increase fruit set, to reduce preharvest drop, to retain fruit firmness during storage, and to inhibit fruit ripening. In vegetable crops, ethylene biosynthesis inhibitors were shown to delay yellowing of broccoli and to induce staminate flowers of cucumber and muskmelon. In flowers, ethylene biosynthesis inhibitors were effective in extending vase life of carnations, snapdragons, daffodils, irises, and chrysanthemums and in reducing flower abscission of snapdragons.

Additional effects and uses of ethylene biosynthesis inhibitors will be found in the future. Hopefully, some of these uses will prove to be economically feasible for commercial practices.

Bibliography

- Adams, D. O., and Yang, S. F., 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Natl. Acad. Sci. USA 76:170-174.
- Amrhein, N., and Wenker, D., 1979. Novel inhibitors of ethylene production in higher plants. Plant and Cell Physiol. 20:1635-1642.
- Apelbaum, A., Burgoon, A. C., Anderson, J. D., Lieberman, M., Ben-Arie, R., and Mattoo, A. K., 1981. Polyamines inhibit biosynthesis of ethylene in higher plant tissue and fruit protoplasts. Plant Physiol. 68:453-456.
- Apelbaum, A., and Icekson, I., 1983. Applied polyamines inhibit macromolecular synthesis in plant tissue. In: Advances in Polyamine Research. Vol 4. Eds. Bachrach, U., Kaye, A., and Chayen, R., Raven Press, New York, p. 437-442.
- Apelbaum, A., Wang, S.Y., Burgoon, A. C., Baker, J. E., and Lieberman, M., 1981. Inhibition of the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene by structural analogs, inhibitors of electron transfer, uncouplers of oxidative phosphorylation, and free radical scavengers. Plant Physiol. 67:74-79.
- Atsmon, D., and Tabbak, C., 1979. Comparative effects of gibberellin, silver nitrate and aminoethoxyvinylglycine on sexual tendency and ethylene evolution in the cucumber plant. Plant and Cell Physiol. 20:1547-1555.
- Autio, W. R., and Bramlage, W. J., 1982. Effects of AVG on maturation, ripening, and storage of apples. J. Amer. Soc. Hort. Sci. 107:1074-1077.
- Baker, J. E., Lieberman, M., and Anderson, J. D., 1978. Inhibition of ethylene production in fruit slices by a rhizobitoxine analog and free radical scavengers. Plant Physiol. 61:886-888.

- Baker, J. E., Wang, C. Y., Lieberman, M., and Hardenburg, R. F., 1977. Delay of senescence in carnations by rhizobitoxin analog and sodium benzoate. *HortScience* 12:38-39.
- Bangerth, F., 1978. The effect of a substituted amino acid on ethylene biosynthesis, respiration, ripening and preharvest drop of apple fruits. *J. Amer. Soc. Hort. Sci.* 103:401-404.
- Ben-Arie, R., Lurie, S., and Mattoo, A. K., 1982. Temperature-dependent inhibitory effects of calcium and spermine on ethylene biosynthesis in apple discs correlate with changes in microsomal membrane viscosity. *Plant Sci. Lett.* 24:239-247.
- Bramlage, W. J., Greene, D. W., Autio, W. R., and McLaughlin, J. M., 1980. Effects of aminoethoxyvinylglycine on internal ethylene concentrations and storage of apples. *J. Amer. Soc. Hort. Sci.* 105:847-851.
- Broun, R., and Mayak, S., 1981. Aminoxyacetic acid as an inhibitor of ethylene synthesis and senescence in carnation flowers. *Sci. Hort.* 15:277-282.
- Burg, S. P., and Thimann, K. V., 1959. The physiology of ethylene formation in apples. *Proc. Natl. Acad. Sci., USA.* 45:335-344.
- Curry, E. A., and Williams, M. W., 1986. Physiological changes associated with increased lateral branching of apple trees treated with aminoethoxyvinylglycine. *J. Amer. Soc. Hort. Sci.* 111:237-240.
- Dekazos, E. D., 1979. Effect of AVG (Aminoethoxyvinylglycine) on bloom delay of peaches. *HortScience* 14:436.
- Dennis, F. G., Jr., Crews, C. E., and Buchanan, D. W., 1977. Bloom delay in stone fruits and apple with rhizobitoxine analogue. *HortScience* 12:386.
- Drolet, G., Dumbroff, E. B., Legge, R. L., and Thompson, J. E., 1986. Radical scavenging properties of polyamines. *Phytochem.* 25:367-371.
- Fuhrer, J., Kaur-Sawhney, R., Shih, L. M., and Galston, A. W., 1982. Effects of exogenous 1,3-diaminopropane and spermidine on senescence of oat leaves. II. Inhibition of ethylene biosynthesis and possible mode of action. *Plant Physiol.* 70:1597-1600.
- Fujino, D. W., Reid, M. S., and Yang, S. F., 1980. Effects of aminoxyacetic acid on postharvest characteristics of carnation. *Acta Hort.* 113:59-64.
- Gane, R., 1934. Production of ethylene by some ripening fruit. *Nature* 134:1008.
- Gepstein, S., and Thimann, K. V., 1981. The role of ethylene in the senescence of oat leaves. *Plant Physiol.* 68:349-354.
- Giovanelli, J., Owens, L. D., and Mudd, S. H., 1971. Mechanism of inhibition of spinach β -cystathionase by rhizobitoxine. *Biochim. Biophys. Acta* 227:671-684.
- Greene, D. W., 1980. Effect of silver nitrate, aminoethoxyvinylglycine, and gibberellins A₄₊₇ plus 6-benzylamino purine on fruit set and development of 'Delicious' apples. *J. Amer. Soc. Hort. Sci.* 105:717-720.
- Greene, D. W., 1983. Some effects of AVG on fruit set, fruit characteristics, and vegetative growth of apple trees. *J. Amer. Soc. Hort. Sci.* 108:410-415.
- Hansen, E., 1942. Quantitative study of ethylene production in relation to respiration of pears. *Bot. Gaz.* 103:543-558.

- John, R. A., Charteris, A., and Fowler, L. J., 1978. The reaction of aminoxyacetate with pyridoxal phosphate-dependent enzymes. *Biochem. J.* 171:771-779.
- Lau, O. L., Murr, D. P., and Yang, S. F., 1974. Effect of 2,4-dinitrophenol on auxin-induced ethylene production and auxin conjugation by mung bean tissue. *Plant Physiol.* 54:182-185.
- Lau, O. L., and Yang, S. F., 1976. Inhibition of ethylene production by cobaltous ion. *Plant Physiol.* 58:114-117.
- Lieberman, M., Kunishi, A. T., and Owens, L. D., 1974. Specific inhibitors of ethylene production as retardants of the ripening process in fruits. In: *Facteurs et Regulation de la Maturation des Fruits. Colloques Int CNRS 238, Paris*, pp. 161-170.
- Loy, J. B., Natti, T. A., Zack, C. D., and Fritts, S. K., 1979. Chemical regulation of sex expression in a gynomonoecious line of muskmelon. *J. Amer. Soc. Hort. Sci.* 104:100-101.
- Murr, D. P., and Yang, S. F., 1975. Inhibition of in vivo conversion of methionine to ethylene by L-canaline and 2,4-dinitrophenol. *Plant Physiol.* 55:79-82.
- Ness, P. J., and Romani, R. J., 1980. Effects of aminoethoxyvinylglycine and countereffects of ethylene on ripening of Bartlett pear fruits. *Plant Physiol.* 65:372-376.
- Owens, K. W., Tolla, G. E., and Peterson, C. E., 1980. Induction of staminate flowers on gynoecious cucumber by aminoethoxyvinylglycine. *HortScience* 15:256-257.
- Owens, L. D., 1969. Toxins in plant disease: structure and mode of action. *Science* 165:18-25.
- Owens, L. D., Lieberman, M., and Kunishi, A. T., 1971. Inhibition of ethylene production by rhizobitoxin. *Plant Physiol.* 48:1-4.
- Richardson, D. G., and Meheriuk, M., 1982. Controlled atmospheres for storage and transport of perishable agricultural commodities. *Proc. 3rd. Natl. CA. Res. Conf.*, Timber Press, Beaverton, OR.
- Sherman, M., 1985. Control of ethylene in the postharvest environment. *HortScience* 20:57-60.
- Shih, L. M., Kaur-Sawhney, R., Fuhrer, J., Samanta, S., and Galston, A. W., 1982. Effects of exogenous 1,3-diaminopropane and spermidine on senescence of oat leaves. I. Inhibition of protease activity, ethylene production, and chlorophyll loss as related to polyamine content. *Plant Physiol.* 70:1592-1596.
- Spencer, M. S., 1959. Ethylene metabolism in tomato fruit. III. Effect of 2,4-dinitrophenol on respiration, ethylene evolution, and ripening. *Can. J. Biochem. Biophys.* 37:53-59.
- Suttle, J. C., 1981. Effect of polyamines on ethylene production. *Phytochem.* 20:1477-1480.
- Wang, C. Y., 1977. Effect of aminoethoxy analog of rhizobitoxine and sodium benzoate on senescence of broccoli. *HortScience* 12:54-56.
- Wang, C. Y., and Baker, J. E., 1979. Vase life of cut flowers treated with rhizobitoxine analogs, sodium benzoate, and isopentenyl adenosine. *HortScience* 14:59-60.
- Wang, C. Y., and Baker, J. E., 1980. Extending vase life of carnations with aminoxyacetic acid, polyamines, EDU, and CCCP. *HortScience* 15:805-806.
- Wang, C. Y., Baker, J. E., Hardenburg, R. E., and Lieberman, M., 1977. Effects of two analogs of rhizobitoxine and sodium

- benzoate on senescence of snapdragons. *J. Amer. Soc. Hort. Sci.* 102:517-520.
- Wang, C. Y., and Mellenthin, W. M., 1977. Effect of aminoethoxy analog of rhizobitoxine on ripening of pears. *Plant Physiol.* 59:546-549.
- Williams, M. W., 1980. Retention of fruit firmness and increase in vegetative growth and fruit set of apples with aminoethoxyvinylglycine. *HortScience* 15:76-77.
- Williams, M. W., 1981. Response of apple trees to aminoethoxyvinylglycine (AVG) with emphasis on apical dominance, fruit set, and mechanism of action of fruit thinning chemicals. *Acta Hort.* 120:137-141.
- Yang, S. F., and Hoffman, N. E., 1984. Ethylene biosynthesis and its regulation in higher plants. *Ann. Rev. Plant Physiol.* 35:155-189.
- Yu, Y. B., Adams, D. O., and Yang, S. F., 1979a. 1-aminocyclopropanecarboxylate synthase, a key enzyme in ethylene biosynthesis. *Arch. Biochem. Biophys.* 198:280-286.
- Yu, Y. B., Adams, D. O., and Yang, S. F., 1979b. Regulation of auxin-induced ethylene production in mung bean hypocotyls. Role of 1-aminocyclopropane-1-carboxylic acid. *Plant Physiol.* 63:589-590.
- Yu, Y. B., Adams, D. O., and Yang, S. F., 1980. Inhibition of ethylene production by 2,4-dinitrophenol and high temperature. *Plant Physiol.* 66:286-290.
- Yu, Y. B., and Yang, S. F., 1979. Auxin-induced ethylene production and its inhibition by aminoethoxyvinylglycine and cobalt ion. *Plant Physiol.* 64:1074-1077.

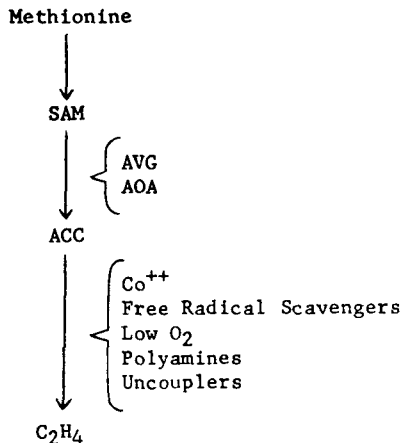


Figure 1 - Steps in the ethylene biosynthetic pathway affected by various inhibitors.