

Effects of Ethylene on Fruit Set and Maturation of Highbush Blueberry (*Vaccinium corymbosum* L.)

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Summary

Flower buds and immature berries in the highbush blueberry 'Jersey' were treated by dipping them in 1-aminocyclopropane-1-carboxylic acid (ACC) or aminooxyacetic acid (AOA) to examine the effects of ethylene on fruit set and maturation. ACC treatment to flower bud 10 days before bloom immediately increased ethylene evolution from flower bud at the stage in which no ethylene evolved in the untreated control bud. The treatment promoted flower and berry abscission indicating that ethylene concentration above the natural level induced flowers and young berries to abscise although ethylene evolution increased 5 days after anthesis. ACC treatment of immature berry also increased ethylene evolution and accelerated maturation. As a result, the harvest was advanced and picking period shortened. AOA treatment as an inhibitor of ethylene evolution did not clearly effect ethylene evolution. Our results indicate that by regulating ethylene evolution, the amount of fruiting and the time of maturation could be controlled.

Introduction

Blueberries (*Vaccinium* spp.) exhibit a typical climacteric respiration curve (Ismail and Kender, 1969; Windus et al., 1976; Lipe, 1978). Windus et al. (1976) reported that blueberries have a climacteric type ripening, and carbon dioxide (CO₂) and ethylene evolution peaked at the green pink (GP) and blue pink (BP) color stages of ripening. That ethylene participates in the maturation of blueberry fruit was reported by Eck (1970) and Warren et al. (1973), who found that 2-chloroethylphosphonic acid (etheal) advanced maturity and the harvest period. But the berries treated by etheal inclined to be small with high acidity and low soluble solids. Dekazos (1979, cited by Eck, 1988) reported that aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis, delays anthesis of the rabbiteye blueberry about 2 weeks and delays the maturation about 1 week, but has no effect on quality.

Applications of gibberellic acid (GA) increased

the percentage of fruit set and promoted parthenocarpic fruit set when pollination by insects was insufficient (Mainland and Eck, 1969a, b; Hooks and Kenworthy, 1971), but GA-treated plants produced smaller berries and prolonged the maturation period (Mainland and Eck, 1969b). Application of GA combined with an auxin, such as 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T) and succinic acid-2, 2-dimethylhydrazide (SADH), increased yield and shortened the harvest period (Doughty and Scheer, 1975).

Controlling maturation by ethylene regulation was not established until recently, but some problems, such as selecting a suitable reagent and determining its concentration and appropriate treatment time remain. Furthermore, there are no reports on the relationship between fruit set and ethylene evolution.

Our previous report (Suzuki et al., 1997) showed that the changes of ethylene evolution during the period of blueberry fruit development, i. e. ethylene evolution peaked at petal-fall and during maturation. The first peak was higher than the second peak; ethylene at petal-fall stage was produced from the ovary and not from the senescing corolla. Ethylene evolution in maturation increased at the middle stage. Ethylene-forming enzyme

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(EFE) activity was similar to that of ACC, but the latter increased during the mature green (MG) to GP stage in peel color. These data indicate that the control of ethylene evolution by ACC regulates ripening of berries.

In this report we discuss the results of our study on the regulation of ethylene evolution and fruit set and maturation.

Materials and Methods

Experiment 1. Effect of ACC or AOA treatment to flower buds on ethylene evolution and flower abscission at the flowering stage.

The experiment was carried out with three high-bush blueberry (*V. corymbosum* L.) cv. 'Jersey' bushes growing in the Iwate Univ. orchard in 1995. Five treatments were prepared to regulate ethylene evolution: (1) 1 mM of ACC, an ethylene precursor in biosynthesis, to promote ethylene evolution; (2) 5 mM of ACC; (3) 1 mM of AOA, an inhibitor of ethylene biosynthesis; (4) 5 mM of AOA; and (5) control. Ten clusters per treatment (approximately one hundred berries) were dipped for 5-10 seconds in a reagent containing spreader (Gramin S, Sankyo Co.) approximately 10 days before anthesis (May 15) and again three days later. Ethylene evolution and the numbers of abscised flowers and berries were measured at regular intervals until 40 days after the first treatment.

Ethylene analysis

Two to ten flowers or berries were placed in a 20-ml bottle and sealed with parafilm for six hours at room temperature. A 1-ml gas sample was taken with a plastic syringe from the bottle, and analyzed by FID-gas chromatography (Shimadzu GC-14A). Each measurement was repeated 3 times.

Experiment 2. Effect of ACC or AOA treatment to immature berries on ethylene evolution, maturing time, and berry quality.

The experiment was carried out with five 'Jersey' bushes growing in the Iwate Univ. orchard in 1995. Five treatments, as in Experiment 1, were prepared to regulate ethylene evolution: (1) 1 mM of ACC, (2) 5 mM of ACC, (3) 1 mM of AOA, (4) 5 mM of AOA, and (5) control. Fifty

clusters were selected per treatment and dipped for 5-10 seconds in a reagent containing spreader (Gramin S, Sankyo Co.) on July 12 and again three days later. The berries on July 12 were at the immature green (IG) stage in peel color; approximately ten days later, they were at the mature green (MG) stage when ripening starts. The color stages classified by Shutak et al. (1980, cited by Gough, 1994) are IG, MG, GP, blue pink (BP), blue (B), and ripe (R). Berries for the ethylene evolution measurement were sampled at each color stage; on July 21 for MG, July 27 for GP and BP and Aug. 3 for B and R. Ethylene evolution was determined as in Experiment 1. The berries at the R stage were picked by hand individually for several days, counted, and weighed. Harvested berries were frozen at -30°C ; 10-g samples were analyzed for soluble solids (SS) and titratable acidity.

Results and Discussion

Experiment 1.

Ethylene evolution (Fig. 1) of the control began to increase 5 days after flowering (DAF) to 14 DAF, and then decreased rapidly. Clusters treated at 1 mM ACC produced ethylene about twice as fast as the control. Moreover, ethylene evolution of the berries treated at 5 mM ACC increased immediately after being dipped; the value was highest at the flower bud stage. At this stage the control bud evolved no ethylene. Ethylene evolution, however, decreased rapidly and there were no difference by petal-fall. AOA treatment at 1 mM and 5 mM did not inhibit ethylene evolution.

Data on bud or flower abscission at the flowering stage (Table 1) show that ACC-treated flowers abscised faster than did those of the control. Of clusters treated with 5 mM ACC, 41.8% abscised which is about ten times that of the control. Additionally, abscission of the bud treated with 5 mM ACC occurred before the flowering stage, whereas no abscission took place at that stage in the control.

Young fruit abscission is one of the effects of ethylene. Flower or fruit abscission is promoted by applying ethylene exogenously to a fruit tree, such as apple (Schneider, 1978), but it is not clear whether ethylene is the primary inducer. There are no reports concerning the relationship between the flower or fruit abscission and internal

ethylene concentration in blueberries.

Our data show that ethylene in an untreated flower evolved between anthesis and petal-fall; nevertheless, flower abscission does not occur, ethylene evolution rate and volume were accelerated by ACC treatment while promoting flower or fruit abscission. These results indicate that

ethylene has no effect on abscission at the natural level (below threshold level); however, abscission is promoted if the level exceeds the threshold value. Accordingly, we could control the number of the fruit set by proving the relationship between ethylene evolution and flower or fruit abscission.

AOA, the inhibitor of ethylene biosynthesis, could not reduce ethylene evolution. Some possible reasons are that the treatment time was unsuitable because ethylene biosynthesis had not begun and/or the AOA was not absorbed or the concentration was not physiologically active.

Experiment 2.

Ethylene evolution of berries at each color stage, a number and quality of the berries picked, and harvest progression are shown in Table 2, Fig. 2, and Fig. 3, respectively. Ethylene evolution of the control was observed at the MG stage, and the value remained constant to the R stage. On the other hand, ethylene evolution of ACC-treated berries was a hundred times higher than that of the control immediately after the first treatment; the rate decreased at the GP stage, but increased at the BP-B stages again. Ethylene evolution of AOA-treated fruits were no different from the control; hence, AOA did not inhibit ethylene biosynthesis.

The cumulative harvests from the first picking till July 27 in the berries treated with 1 mM and 5 mM ACC were 52.5% and 65.9%, respectively, whereas that of the control was 13.7%. The results indicate that the acceleration of time and volume of ethylene hastened the ripening of berry. Berry weight and SS tended to be higher and titratable acidity lower in the fruit treated with 1 mM ACC than those of the control, but the same tendency was not observed in the fruit treated with 5 mM ACC.

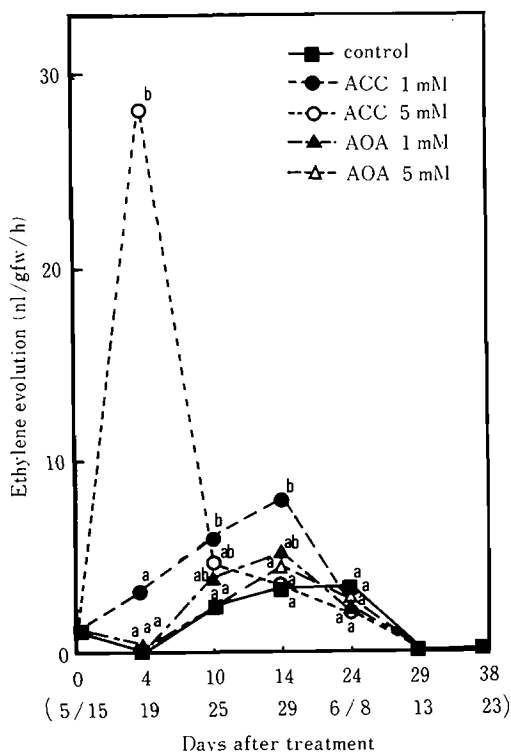


Fig. 1. Ethylene production of flower buds and flowers treated by ACC or AOA before bloom. () shows calendar days. Full bloom was May 25. Mean separation in each treatments by F-test, 5% level.

Table 1. Effect of ACC, AOA applications on flower buds on flower or young berry abscission.

| Treatment | Number of berries | Percent of abscission (%) | | | | | | | |
|-----------|-------------------|---------------------------|-----|-----|-----|-----|-----|-----|-------|
| | | 5/19 | 30 | 6/8 | 14 | 21 | 29 | 7/7 | Total |
| Control | 100 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 1.0 | 2.0 | 4.0 |
| ACC 1 mM | 83 | 0.0 | 0.0 | 1.2 | 2.4 | 1.2 | 6.0 | 2.4 | 13.2 |
| ACC 5 mM | 103 | 3.9 | 8.7 | 9.7 | 7.8 | 1.9 | 8.7 | 1.0 | 41.8 |
| AOA 1 mM | 90 | 0.0 | 0.0 | 0.0 | 0.0 | 1.1 | 0.0 | 1.1 | 2.2 |
| AOA 5 mM | 82 | 0.0 | 0.0 | 0.0 | 1.2 | 1.2 | 4.9 | 0.0 | 7.3 |

Table 2. Effect of ACC, AOA applications to immature berries on ripening, berry weight, and quality.

| Treatment | Number of berries | Percent of picking (%) | | | | | | | Lost ^z |
|-----------|-------------------|------------------------|------|------|------|------|------|-----|-------------------|
| | | 7/21 | 27 | 8/3 | 9 | 17 | 25 | 25 | |
| Control | 95 | 0.0 | 13.7 | 25.3 | 18.9 | 26.3 | 14.7 | 1.1 | |
| ACC 1 mM | 78 | 11.5 | 41.0 | 14.1 | 15.4 | 11.5 | 3.8 | 2.6 | |
| ACC 5 mM | 88 | 30.7 | 35.2 | 18.2 | 9.1 | 5.7 | 0.0 | 1.1 | |
| AOA 1 mM | 95 | 1.1 | 26.3 | 31.6 | 15.8 | 20.0 | 5.3 | 0.0 | |
| AOA 5 mM | 81 | 0.0 | 0.0 | 19.8 | 19.8 | 30.9 | 29.6 | 0.0 | |

| Treatment | Berry weight (g) | | | | | | ave. | Soluble solids ^y (Brix) | Titratable acidity ^y (g/100g) |
|-----------|------------------|------|------|------|------|------|------|------------------------------------|--|
| | 7/21 | 27 | 8/3 | 9 | 17 | 25 | | | |
| Control | - | 1.16 | 0.94 | 0.88 | 0.73 | 0.73 | 0.87 | 10.8 | 1.17 |
| ACC 1 mM | 1.28 | 1.27 | 1.05 | 0.82 | 0.73 | 0.74 | 1.08 | 12.4 | 0.76 |
| ACC 5 mM | 1.10 | 1.02 | 1.12 | 1.22 | 0.87 | - | 1.07 | 9.2 | 1.06 |
| AOA 1 mM | 1.05 | 1.01 | 1.02 | 0.83 | 0.75 | 0.77 | 0.98 | 10.8 | 1.11 |
| AOA 5 mM | - | - | 0.94 | 0.79 | 0.79 | 0.76 | 0.83 | 10.3 | 1.41 |

^z Dropped berries for accident except picking.

^y Berries were sampled at July 27 and Aug. 3 (except AOA 5 mM), at Aug. 3 and 9 (AOA 5 mM).

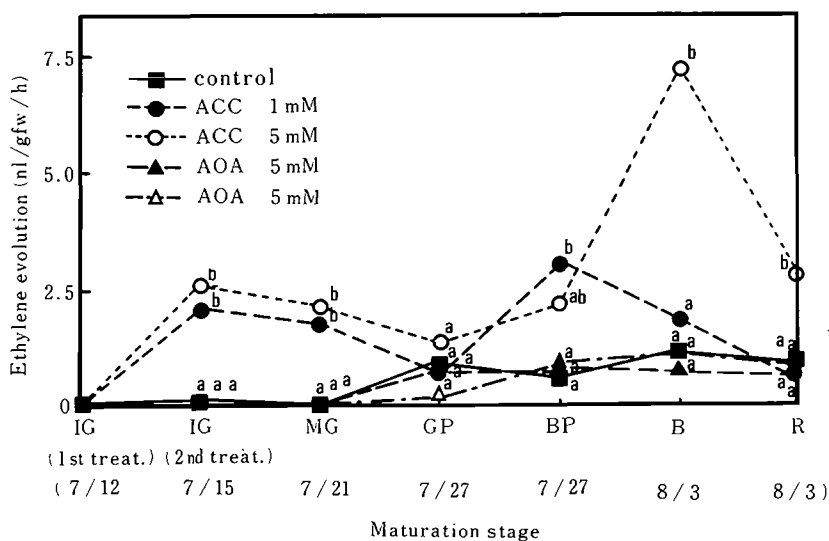


Fig. 2. Ethylene production of berries treated by ACC or AOA before maturation. Maturation stages; immature green (IG), mature green (MG), green pink (GP), blue pink (BP), blue (B) and ripe (R).

() shows calendar days treated or sampled. Mean separation in each treatments by F-test, 5% level.

As ACC is the precursor in ethylene biosynthesis (Lizada and Yang, 1979) so that ACC applied exogenously would accelerate ethylene evolution and advance berry ripening was predictable. We

conclude that acceleration of the time in ethylene evolution triggered early ripening.

Concerning berry quality, Warren et al. (1973) reported that berry weight and SS decreased and

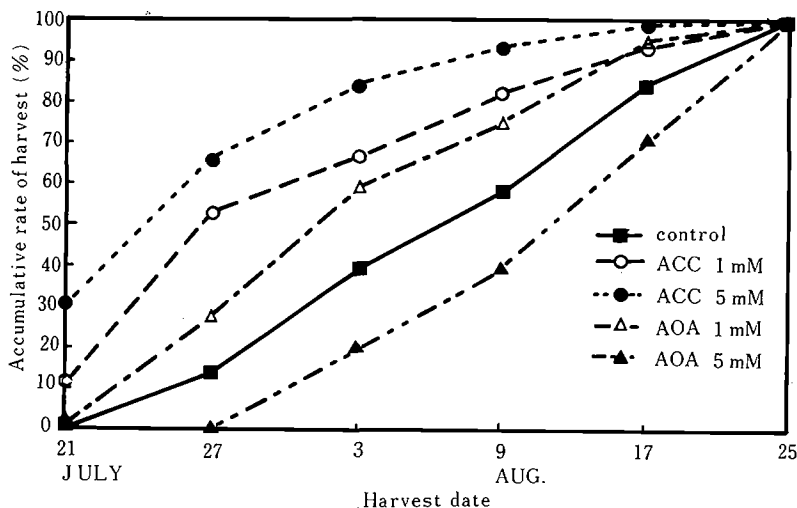


Fig. 3. Harvest rate of berries treated by ACC or AOA before maturation. Berries were treated on July 12 (1st. treatment) and July 15 (2nd. treatment).

acidity increased with increasing concentrations of ethrel. They also indicated that the optimum time of application was near the end of Stage II and the beginning of Stage III. The quality of 1 mM ACC-treated berries was superior to that of the control. Because the time of our treatment corresponded to the end of Stage II, ACC concentration may have been optimum. Additionally, the quality of berries of 5 mM ACC tended to be poorer than that of the control, similar to the that of Warren et al. (1973). Thus, we recognize that the difference in the volume of ethylene evolution has considerable influence on the time of maturation and berry quality.

AOA treatment did not decrease ethylene evolution as well as we expected. However, with 5 mM AOA treatment, the ripening of berries was delayed; ethylene evolution was not inhibited, and the titratable acidity was higher than that of the control. We have no explanation as to why 5 mM AOA treatment delayed the maturation of berry without decreasing of ethylene evolution. Dekazos (1979, cited by Eck, 1988) reports that AVG delays the maturation of the rabbiteye blueberry about 1 week, so that it is possible to regulate ethylene evolution and berry maturation by AOA treatment. However, berries late ripen tend to result in small and poor quality fruits (Yokota, 1989), so that those techniques are unsuitable at

present.

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ハイブッシュブルーベリーの結実および成熟に対するエチレンの影響

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摘 要

ブルーベリー果実の開花期および成熟期におけるエチレン生成が結実および成熟に及ぼす影響を検討するため、ハイブッシュブルーベリー 'Jersey' の花らいと成熟前果実に ACC および AOA を浸漬処理した。開花 10 日前の花らいに ACC を処理すると、エチレン生成は無処理区では生成しない花らい時から急激に高まり、落花・果を促進した。無処理区では満開 5 日後頃にエチレンを生成したが、落花はほとんど生じなかったことから、あるいき値以上のエチレン生成量で落花

・果が促進されることが認められた。成熟前の果実に ACC を処理すると、エチレン生成を高めるとともに成熟を促進した。その結果、早期収穫率が高まり、収穫期間が短縮された。エチレン生成抑制剤として処理した AOA は開花期および成熟期とも明確な抑制効果がみられなかった。以上の結果はエチレン生成の制御により結実量および成熟時期を調節できる可能性を明らかにした。