J. Japan. Soc. Hort. Sci. 66(1) : 23-27. 1997.

Changes of Ethylene Evolution, ACC Content, Ethylene Forming Enzyme Activity and Respiration in Fruits of Highbush Blueberry

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Summary

We measured ethylene evolution, ACC content, ethylene forming enzyme (EFE) activity, and respiration in highbush blueberry (*Vaccinium corymbosum* L.) from flowering to harvest to assess the role of ethylene in the processes. Cultivars used were 'Collins' (early maturing), 'Berkeley' (mid-season), and 'Dixi' (late maturing). Ethylene evolution increased at both petal-fall and maturation stages, and was higher in the early stage than in the late stage. Ethylene at petal fall was produced by the calyx and ovary parts, but not the corolla. This indicates that ethylene evolution in flowering is correlated with pollination or fertilization or both. Ethylene evolution rate in the maturation stage was high between the blue pink (BP) peel color in 'Collins', and mature green (MG) stage in 'Berkeley', and green pink (GP) stage in 'Dixi'. Ethylene evolution was higher for 'Collins' than for the others in both years and higher in 1995 than in 1994. The pattern of EFE activity was not similar to that of ethylene evolution except in 'Collins'. Therefore, the correlation between ethylene evolution and EFE activity in the maturation stage was different with each cultivar. Berry ACC content was high at GP stage in three cultivars before ethylene evolution increased.

Introduction

Blueberry (*Vaccinium* spp.) is classified as a berry, and the period from the time the petals drop until the berry ripens is 50 to 60 days on average (Shutak and Marucci, 1966). During this period, the berry growth curve follows a double sigmoid like the peach and grape; maturation occurs at Stage III. However, each individual berry does not ripen uniformly on a cluster, so that a grower must harvest each ripe berry by hand. The harvest period takes two to three weeks so that the picking cost is the most expensive in blueberry culture. The growers are expecting a physico-chemical control of maturation. Therefore, we seek to clarify the physiological regulation of fruit maturation.

Ismail and Kender (1969) first reported that blueberries exhibit a climacteric rise in respiration in a greenhouse. Windus et al. (1976) reported that blueberries have a climacteric type ripening; carbon dioxide (CO_2) and ethylene evolution peaked during the middle stage of ripening. However, there are only a few reports (Eck, 1970; Warren et al., 1973) about the regulation of ripening by controlling ethylene evolution. No report was found regarding the change of 1-aminocyclopropane-1-carboxylic acid (ACC) and the ethylene-forming enzyme (EFE) activity in ethylene biosynthesis.

In this research, we measured ethylene evolution during flowering to ripening, ACC content, EFE activity, and respiration during the maturation stage. The relationships between these ethylene evolution factors and fruiting or maturation are discussed.

Materials and Methods

Highbush blueberries (*V. corymbosum* L.) cv. 'Collins', 'Berkeley', and 'Dixi' growing in the Iwate Univ. orchard were utilized for this study in 1994 and 1995. Swollen flower buds, flowers in bloom, and the developing berry were sampled at

Received; May 31, 1996. Accepted; August 13, 1996.
A part of this paper was presented at the 1996 Spring Meeting of the Japanese Society for Horticultural Science.
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Table 1. Color grade during berry maturation.

Color stage		Description	Days before harvest
Mature green	(MG)	light green	8
Green pink	(GP)	Some pink showing at calyx	6
Blue pink	(BP)	Mostly blue, some pink at stem end	4
Blue	(B)	Nearly blue, pink ring at stem	2
Ripe	(R)	All blue	0

5- to 10-day intervals. These samples were weighed and their ethylene evolution rates measured. Berries, during the maturation stage, were colected at different colored stages as classified by Shutak et al. (1980, cited by Gough, 1994) (Table 1). Their ethylene and CO_2 evolution rates, ACC content, EFE activity determined. Abbreviation of color stages are: mature green (MG), green pink (GP), blue pink (BP), blue (B), and ripe (R). The maturation periods of these three cultivars in the Iwate area ranged from early July for 'Collins', middle-late July for 'Berkeley', and early August for 'Dixi'.

Ethylene analysis

Two to ten flowers or berries were placed in a 20-ml bottle and sealed with para film (American can Co.) for six hours at 20-25 °C. A 1-ml gas sample was taken from the bottle with a plastic syringe and analyzed by FID-gas chromatography (Shimadzu GC-14 A). Each measurement was repeated at least three times.

ACC analysis

Five g of berries from each color stage were homogenized in 10 ml of 5% sulfosalicylic acid using an ultra homogenizer. After centrifuging the homogenate, the supernatant was passed through ion-exchange resin (Dowex 50 X-8, H^+), which then was eluted with water. The ACC which was eluted from the column with 2 N NH_4OH was determined by the method of Lizada and Yang (1979). Each measurement was repeated at least three times.

EFE activity

Two berries were diced into eight pieces and incubated at 30 °C in a 30-ml vial containing 5 ml of incubation medium including 5 mM ACC and 0.5 M sorbitol in 1/30 M phosphate buffer adjusted to pH 5.5. After incubating for two hours, a 1-ml gas sample was analyzed for ethylene as above. EFE activity (the ability to convert ACC to ethylene) was expressed as nl ethylene/gfw/h. Each measurement was repeated at least three times.

Respiration

A 1-ml gas sample was taken from the same bottle as that for ethylene analysis, and its CO_2 content determined by TCD-gas chromatography. Each measurement was repeated at least three times.

Results and Discussion

Fig. 1 shows ethylene evolution in flower bud, flower, and berry from bud swell to harvest in three cultivars. Ethylene evolution peaked at the petal-fall stage, 5 or 6 days after flowering in all cultivars. After that, ethylene evolution decreased rapidly and only trace amounts were found in the developing berry until maturation started. The second peak of ethylene evolution was found at mid-stage of maturation. This peak, however, was lower than that of the petal-fall stage. Ethylene evolution at the petal-fall stage was higher for 'Berkeley' in 1994, and for 'Collins' in 1995 than the other cultivar. Ethylene evolution rate at maturation was fastest in 'Collins' in both years and higher in 1995 than in 1994.

A peak of ethylene evolution was recorded at petal fall and at fruit maturity; the first peak was higher than the second. Shimura et al. (1986) did not show the early peak, because their measurements were started 17 days after pollination. Lipe (1978) reported that ethylene evolution peaked approximately two weeks after bloom and again at a lower rate as fruits approached harvest. He also found no correlation between the post-bloom ethylene peak and the abscission of immature fruit. In our study, the first peak occurred at

petal-fall stage, much earlier than that of Lipe (1978). Therefore, we surmise that ethylene was produced from senescing petals. Ethylene evolution rates of isolated corolla, calyx, and ovary disclosed that ethylene evolution was higher in the calyx and ovary than in the corolla (Table 2). That ethylene evolution at post-bloom did not correlate with immature fruit abscission was reported by Lipe (1978); and that ethylene biosynthesis was higher in style, ovary, and receptacle tissues than in petals (Woodson et al., 1992), Nadeau et al. (1993) showed that ACC oxidase activity increased after pollination. Therefore, ethylene evolution may be attributable to physiological changes during pollination or fertilization or both and not to petal senescence.

The difference in ethylene evolution of maturation stage between 1994 and 1995 may be attributed to the high temperatures in the summer season of 1994, when ripening was advanced by about one week. However, we could not account for the difference during the petal-fall stage.

Fig. 2 shows the change of ethylene evolution,

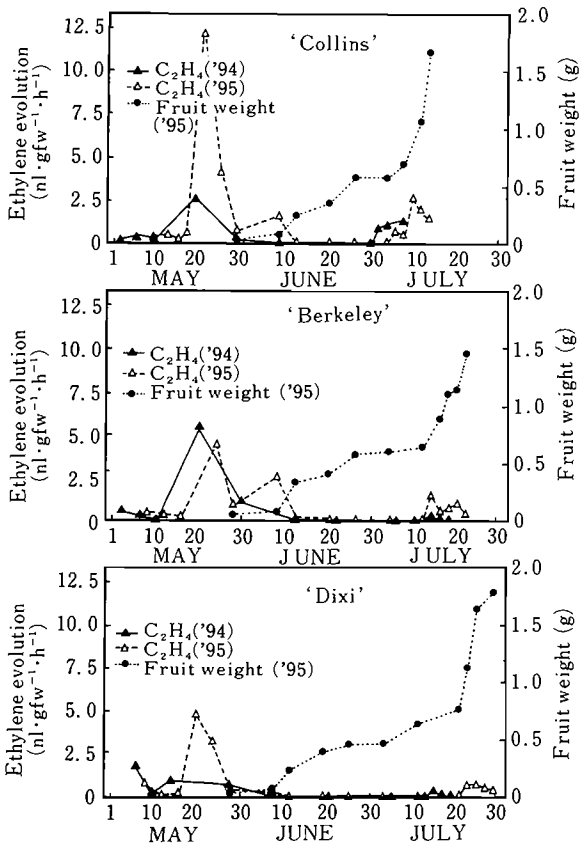

Fig. 1. Change of ethylene evolution from anthesis to harvest, and fruit growth curve in three blueberry cultivars.

Table 2. Ethylene evolution rate by different flower parts.

Cultivar	Part	Ethylene evolution (nl/gfw/h)	
		Blooming	Petal fall
Collins	Petal	0.34	1.15
	Calyx·Ovary	1.94	14.65
Berkeley	Petal	0.26	1.29
	Calyx·Ovary	1.68	10.64
Dixi	Petal	tr.	0.17
	Calyx·Ovary	2.11	13.44

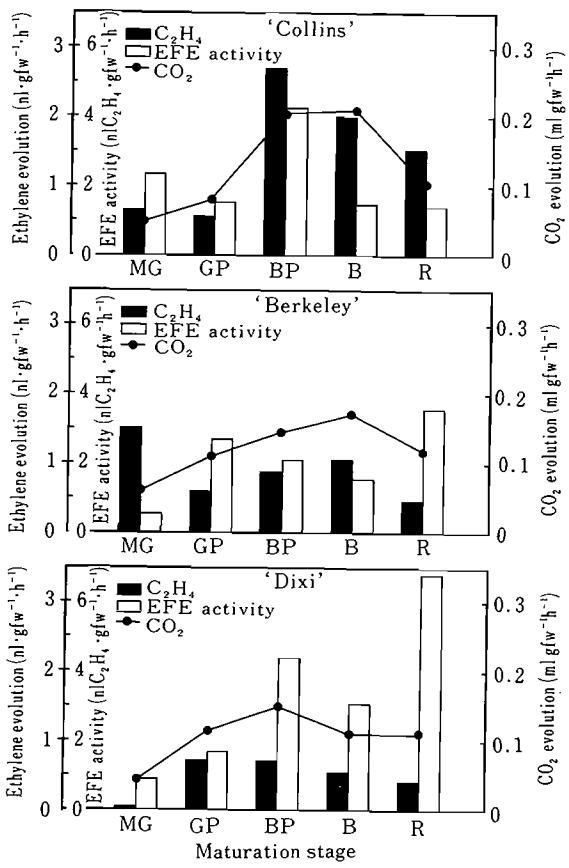

Fig. 2. Change of ethylene and CO_2 evolution rates, and EFE activity during maturation in three blueberry cultivars. Maturation stage; Mature green (MG), Green pink (GP), Blue pink (BP), Blue (B), Ripe (R).

Table 3. Changes of ACC content during maturation.

Cultivar	ACC content (nmol/gfw)				
Maturation stage	MG	GP	BP	B	R
Collins	0.08	0.18	0.17	0.08	0.10
Berkeley	0.25	0.37	0.17	0.17	0.16
Dixi	0.25	0.33	0.20	0.13	0.13

Maturation stage; Mature green (MG), Green pink (GP), Blue pink (BP), Blue (B), Ripe (R).

EFE activity and respiration during maturation stage in three cultivars. Ethylene evolution increased from GP to BP stages of maturation in 'Collins', from MG to GP stage in 'Dixi', but these patterns were not apparent in 'Berkeley'. EFE activity increased almost paralleling the ethylene evolution in 'Collins', but in 'Dixi' the high activity of EFE continued even though rate of ethylene evolution decreased. Respiration activity also increased with ethylene evolution. 'Collins' has high ethylene evolution and respiration rates. The ACC content of the berry was high at the GP stage before ethylene evolution increased for the three cultivars (Table 3).

Windus et al. (1976) determined the rate of respiration and ethylene evolution in each color grade of maturation stage in highbush blueberry of seven cultivars, and found that the rate of respiration of the berries generally increased from the IG to a peak level at the GP or BP stage and that ethylene evolution attained a peak at GP. They concluded that the blueberry should be classified as a climacteric type fruit to which we agree. However, if the blueberry is a climacteric type fruit, ethylene evolution should start before the MG stage.

The rise in EFE activity coincided with that of ethylene evolution in 'Collins', but, it did not in other cultivars. In 'Dixi' fruit, ethylene evolution was lower than those of other cultivars, whereas EFE activity was high. ACC content was high at the MG to GP stage, which correspond to the stage before ethylene evolution increased. The ACC content was lower in 'Collins' than in the other cultivars; it decreased at a later stage of maturation in all cultivars, possibly because ACC was metabolized to ethylene. We could not clarify the high correlation between ethylene evolution and EFE activity during fruit maturation periods.

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ハイブッシュブルーベリー果実のエチレン生成量，ACC 含量，エチレン生成酵素活性および呼吸活性の変化

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摘　要

ブルーベリー果実のエチレン生成と結実および成熟との関係を明らかにするため，開花から成熟に至るまでのエチレン生成量，ACC 含量，EFE 活性および呼吸活性の変化を測定した．エチレン生成は花弁落下期と成熟期に増加し，その量は花弁落下期の方が多かった．花弁落下期のエチレンは，がく・子房部分から生成しており，受粉・受精との関連が示唆された．成熟期のエチレンを着色ステージ別に測定した結果，エチレン生成は 'Collins' では果皮色の blue pink (BP) 期で，'Berkeley' では mature green (MG) 期で，'Dixi' では green pink (GP) 期で高かった．EFE 活性は 'Collins' ではエチレン生成時期と一致したが，他の品種では異なり，成熟期のエチレン生成と EFE 活性の関係は品種により異なった．ACC 含量は 3 品種とも GP 期に多く，成熟終期には低下した．