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Ethephon Application Induces Symptoms of Fruit Tissue Degeneration in Watermelon

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Flesh tissue degeneration (called blood-black heart; BBH) of watermelon (*Citrullus vulgaris* S.) is occasionally observed in Korea. Fruits with BBH have lower quality, a dark-red flesh, and reduced firmness of the rind, often producing an unpleasant flavor. Although causal factors are thought to be undesirable soil moisture conditions, e.g., drought or water-logging, temperature extremes, or virus infection, the mechanism for this physiological disorder is not clearly understood. It is possible that ethylene gas (C_2H_4) is involved in degrading the cell walls. To determine such an implication for ethylene in the occurrence of BBH, we foliar-applied various concentrations of ethephon (100, 200, 400, 800, or 1000 mg L^{-1}) to watermelon plants at 5 or 10 d prior to harvesting, then monitored the development of this disorder in their fruits. At 400 mg L^{-1} or higher, quality was diminished and the fruit had softer rind tissues. About 25% of the fruits harvested at that level exhibited BBH versus 100% of the fruits exposed to 800 or 1000 mg L^{-1} ethephon. Concomitant with the onset of BBH, those affected fruits produced elevated amounts of ethylene gas during the 4-d measurement period. Therefore, a high incidence of BBH may well be related to this increased ethylene production, which can be triggered by both unfavorable environmental conditions and inappropriate cultural practices.

Keywords: blood-black heart (BBH) symptoms, cell wall, ethylene evolution, fruit softening, rind thickness, soluble solids

Watermelon (*Citrullus vulgaris*) is one of the most popular fruit-vegetable crops in South Korea, with a total annual growing area of 2×10^4 ha (7.8×10^5 tons produced) and $800 million in wholesale value each year (MAF, 2006). However, the collapse of flesh tissues inside the ripe fruit is becoming increasingly more prevalent, especially under unfavorable environmental conditions. Such tissues are manifested by a dark-red color resembling "blood" and an unpleasant odor. Initially, the flesh around the seed appears water-soaked and dull-colored before gradually turning dark red or dark pink. The external skin of the fruit loses resilience and becomes less shiny. Ripe fruits sound hollow when knocked on during harvest. Korean growers call this type of fruit "blood-clot" or "blood-black heart (BBH)" watermelon, which has a low sugar content and no market quality. Although the cause for this abnormality is not well understood, undesirable soil moisture conditions, e.g., drought and waterlogging, temperature extremes, and infection by cucumber green mottle mosaic virus (CGMMV) are suspected to be contributing factors for this physiological disorder (Lee et al., 1998).

Park et al. (2000) have reported that covering individual fruits with newspaper or shade cloth to block intense sunlight during ripening reduces the incidence of BBH. Symptoms develop more readily when plants are grown in sandy soil or exposed to a low soil moisture content for 20 d after pollination (Park et al., 1999). A similar problem, "fermented fruits", occurs in Oriental melon (*Cucumis melo*), where levels of acetaldehyde, ethanol, and ethyl acetate increase in tissues when fruits are exposed to hypoxic conditions for 48 h (Suh et al., 1998). Plants grown under low temperature, excessive soil moisture, low light intensity, or excessive nitrogen produce low-quality fruits that eventually show "fermented fruit" characteristics (Choi, 1998; Chung et al., 1998; Hwang and Lee, 1993; Shin et al., 1991). Fruits exposed to ethylene have accelerated tissue softening and increased concentrations of fructose and sucrose after 3 d of treatment (Bianco and Pratt, 1977). This softening is a result of the formation of soluble pectin from protopectin during storage (Cohen and Hicks, 1986). At 1000 ppm, ethylene also promotes tissue softening and cell wall disintegration in persimmon fruits (*Diospyros kaki*) (Park et al., 2003). The objective of our study was to investigate the cause of BBH and the role of ethylene in tissue softening and degeneration of watermelon fruit.

MATERIALS AND METHODS

Plant Materials and Growing Conditions

Seeds of watermelon (*Citrullus vulgaris* cv. Sambockgul; Seminis Korea Co., Seoul, Korea) were sown in plastic plug trays (50×30×7 cm) containing a peat-lite root substrate (Barokuh; Seoul Agric. Supplies, Seoul). Seeds of gourd (*Lagenaria siceraria* cv. FR-Dantos; Syngenta-Korea, Seoul) were germinated in the same substrate as above. When the melon seedlings showed the tips of their first true leaves, we performed grafts using 'Sambockgul' as the scion and 'FR-Dantos' as the rootstock. After 30 d, the grafted plants were placed into soil in a polyethylene film-covered greenhouse (7 m×35 m), at a spacing of 60 cm, with rows 3 m apart. At the six-true-leaf stage, all vines except two, per plant, were pinched to promote branching. At anthesis, the female flowers on the 11th node were self-pollinated, using more than two staminate flowers per pistil. To stimulate fruit enlargement, ovaries were coated with a forchloro-fenurone solution containing 50 μg g^{-1} of CPPU [1-(2-chlor-4-pyridyl) 3-

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phenylurea]. For each plant, only one pollinated flower was left on each of the two branches; all other female flowers were removed.

Ethephon Application and Measurement of Ethylene Evolution

Plants were treated with ethephon (39% active ingredient, 2-chloroethyl phosphonic acid) at 10 or 5 d prior to harvest. A concentration of 100, 200, 400, 800, or 1000 mg L^{-1} was applied with a hand sprayer, to run-off, to the leaf on the fruit-bearing node. No ethephon was applied to the control plants. The temperature was held at 25±2°C during the treatment period. The percentage of BBH occurrence, soluble solid content, fruit firmness, and rind thickness were determined after harvest. Soluble solid contents were measured by a refractometer (Model PR-101; Atago Co., Tokyo, Japan); firmness, with a fruit firmness analyzer (FHM-5, Japan). For plants treated with ethephon at 5 d before harvest, each of their two fruits was put into an 18-L plastic box and sealed completely. Over a 4-d course, the amount of ethylene that evolved inside the container was measured by taking air samples with a hypodermic syringe and analyzing them via gas chromatography (Hewlett-Packard 5890; USA). Conditions included a packed analytical column (2 m×2 mm i.d., active alumina 60/80 mesh), an injector temperature of 110°C, a column temperature of 70°C, and a detector temperature of 150°C. The carrier gas was helium (99.9%) at a flow rate of 30 mL min^{-1}.

Experimental Design and Data Analysis

Our completely randomized plot design comprised 10 treatments (5 ethephon concentrations X 2 application times), with locations randomly assigned in each of three greenhouses. Concentration was the main block, with five plants per spray treatment (three replications). Environmental conditions, such as light intensity, humidity, and temperature, were similar among greenhouses, and cultural practices were uniform for all plants. For each treatment, the measurement unit was an average of three fruit samples. Quality data were subjected to an analysis of variance (ANOVA), and mean values were separated according to least significant difference (LSD) tests at $p=0.05$.

RESULTS

When plants were sprayed 5 d before harvest, higher ethephon concentrations were correlated with thinner and less-firm rinds, whereas the soluble solid content of the fruit was unaffected by treatment (Table 1). A similar but less-pronounced trend was found when ethephon was applied 10 d before harvest. Applications stimulated the occurrence of BBH, especially at higher concentrations. For example, symptoms were induced when a concentration of at least 400 mg L^{-1} was applied 5 d prior. When treatment was delivered 10 d beforehand, all concentrations caused BBH, with 11% of the fruits showing this symptom at the lowest ethephon level (200 mg L^{-1}). As the concentration increased, a greater percentage of the fruit exhibited BBH after either application time, although more symptoms developed when plants were sprayed at 10 d before harvest.

Table 2 summarizes the evolution of ethylene gas when plants were treated 5 d prior to harvest. At 1000 mg L^{-1} ethephon, fruits produced 54 μL kg^{-1} h^{-1} for the first 2 h, and 120 μL kg^{-1} h^{-1} over 24 h. As time passed, the amount of released ethylene gradually decreased to 57 μL kg^{-1} h^{-1} at 96 h. Fruits from plants that had received 800 or 400 mg L^{-1} ethephon emitted the largest amount of ethylene, 72 or 38 μL kg^{-1} h^{-1}, respectively, after 24 h. Those from plants

Table 1. The effect of ethephon applications on changes in watermelon fruit quality and the occurrence of blood-black heart (BBH) symptoms.

Ethephon conc. (mg L^{-1})	Rind thickness (mm)	Rind firmness (kg per 5 mmØ)	Soluble solid content (%)	Occurrence of BBH (%)
Applied 5 days before harvest				
100	13.2 ab[z]	3.05 ab	9.7 abc	0 e
200	13.6 ab	2.80 bc	9.4 abc	0 e
400	12.9 ab	2.75 bc	10.0 a	25.0 d
800	11.5 bcd	2.69 bcd	9.4 abc	62.5 bc
1000	12.4 bc	2.54 cd	10.0 a	85.7 ab
Regression	L*, Q*	L***, Q***	LNS, QNS	---
Applied 10 days before harvest				
100	12.6 b	2.78 bc	9.9 ab	0 e
200	12.8 b	2.66 bcd	9.5 abc	11.1 d
400	12.8 b	2.41 cd	9.3 bc	85.7 ab
800	9.7 d	2.31 d	9.5 abc	100 a
1000	9.9 cd	2.53 cd	9.1 c	100 a
Control	15.5 a	3.41 a	9.4 abc	0 e
Regression	L***, Q**	L*, Q***	LNS, QNS	---

[z]Means separation by least significant differences (LSD) test at $p=0.05$.
Regression analysis: Among curve fittings of linear, quadratic, or cubic, the regression with the highest F value was expressed.

Table 2. Evolution of ethylene from watermelon fruits over a 96-h time course. Various concentrations of ethephon were applied to plants 5 d before harvest.

Ethephon conc. (mg L^{-1})	Ethylene evolved (µL kg^{-1} h^{-1})[z]					Regression	R^2
	2 h	24 h	48 h	72 h	96 h		
0	0	0	0	0	0.1	L^{NS}	0.226
100	0	11.1	9.2	9.5	6.4	Q**	0.741
200	4.2	6.7	5.0	3.7	3.3	Q**	0.922
400	25.6	37.8	32.5	29.1	20.9	Q**	0.847
800	42.3	72.1	37.9	32.1	26.7	Q*	0.819
1000	54.0	120.0	76.2	71.8	57.1	Q*	0.775
$LSD_{0.05}$	4.21	4.19	3.14	1.92	2.03		
Regression	L***	L***	L***	L***	L***		
R^2	0.972	0.955	0.893	0.861	0.887		

[z]Average values from 2 fruits used for measuring evolved ethylene.
Regression analysis: Among curve fittings of linear, quadratic, or cubic, the regression with the highest F value was expressed.

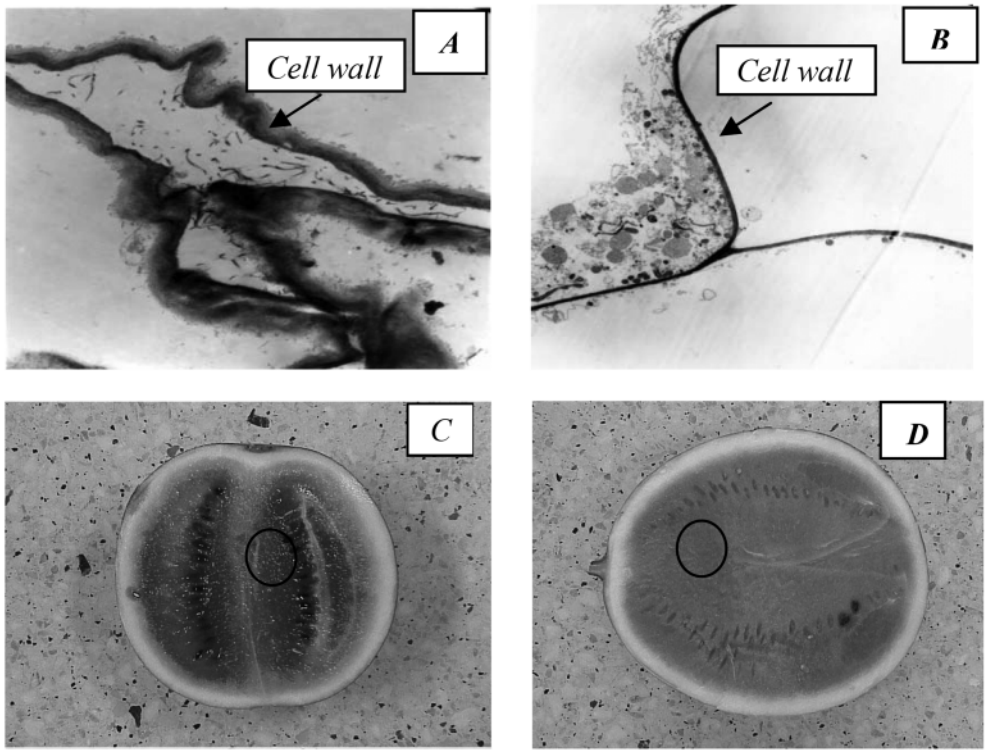

Figure 1. Influence of foliar 1000 mg L^{-1} ethephon application on cell wall integrity and flesh tissue degeneration in watermelon. (**A**) TEM micrograph of transverse section showing disintegration of cell walls in fruit with BBH. Bar = 0.5 µm. (**B**) TEM micrograph of transverse section of cell walls from unaffected control. Bar = 0.5 µm. (**C**) Typical BBH symptoms. (**D**) Unaffected control. **A** and **B** are magnifications of circled regions in **C** and **D**, respectively.

treated with 200 or 100 mg·L^{-1} ethephon evolved less than 11 µL kg^{-1} h^{-1} over the 96-h experimental period.

The most striking response to ethephon application was the appearance of blood-black heart symptoms. Affected fruits had an unusual dark-red flesh color and more lignified, tough tissues that drastically reduced their taste and palatability (Fig. 1). Especially when symptoms were induced by at least 400 mg L^{-1} ethephon, fruits were unsuitable for eating mainly because of an unpleasant odor and tissue degeneration.

DISCUSSION

Foliar application of ethephon was detrimental to the quality of our watermelon fruits, making the rind thinner and softer as the concentration was increased. However, their soluble solid contents were unaffected by treatment. This negative influence was more evident when ethephon was applied 10 d before harvest. Our results are similar to earlier findings that ethephon makes the rind tissue of muskmelon thin and soft (Bianco and Pratt, 1977). Park et al.

(2003) also have reported that, when persimmon is treated with 1000 mg L^{-1} ethephon, fruit firmness is reduced because of softer cell walls (Park et al., 2003).

In general, greater amounts of ethylene were released from fruit tissues as the concentration of ethephon increased. Similar results have been reported by Choi et al. (2001) in ethephon-treated muskmelons grown either hydroponically or in soil. Although ethylene evolution was greatly reduced 5 d after storage in our study, the affected fruits were sometimes not edible. Here, watermelons harvested from plants treated with 400 mg L^{-1} ethephon were unsuitable mainly because of the bad odor associated with BBH. However, those from plants exposed to 200 or 100 mg L^{-1} ethephon were edible.

BBH symptoms that appeared after ethephon application were characterized by their dark-red, tough flesh. Unlike from the untreated plants, cell walls of the affected fruit collapsed and became nonfunctional. This response has also been found by Park et al. (2003) in 'Cheongdobansi' persimmon.

Our observations suggest that ethylene production from fruit tissues is closely related to the onset of BBH in watermelon. Here, cell wall properties were altered and the enhanced ethylene production accelerated the occurrence of BBH. A greater incidence of watermelons with BBH has previously been noted under stressful environmental conditions, e.g., low light, drought, and water-logging, and may well explain the involvement of ethylene in this physiological disorder. A higher percentage of watermelons developed BBH symptoms when plants were stressed during the early fruit-ripening period than at harvest time. Hence, for high-quality watermelon production, we strongly recommend that growers avoid any environmental or biotic stress that can trigger ethylene evolution during fruit ripening.

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