Ethylene Evolution by Tomatoes Stressed by Ammonium Nutrition

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Abstract. Studies of ethylene evolution by tomato (*Lycopersicon esculentum* Mill.) fruit were conducted with plants receiving $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$ nutrition. Fruit of plants grown with $\text{NH}_4\text{-N}$ had a higher occurrence of blossom-end rot (BER), higher $\text{NH}_4\text{-N}$ concentrations, and higher ethylene evolution rates than fruit from plants grown with $\text{NO}_3\text{-N}$. Fruit of plants grown with $\text{NO}_3\text{-N}$ showed no enhancement in ethylene evolution with BER development. Fertilizing these plants with $\text{Ca}(\text{NO}_3)_2$ doubled the average Ca concentration of fruit and restricted BER development. Ammonium suppressed Ca accumulation in fruit relative to those grown with $\text{NO}_3\text{-N}$. Ethylene evolution increased as fruit from plants receiving $\text{NO}_3\text{-N}$ ripened, but without a concomitant increase in $\text{NH}_4\text{-N}$ concentrations in the fruit. Ammonium accumulation in fruit induced BER and enhanced ethylene evolution. These relationships were unique, for $\text{NH}_4\text{-N}$ accumulation did not seem to be a naturally occurring phenomenon in ripening fruit or in fruit that have BER arising from other causal factors.

Blossom-end rot (BER) of fruit is a physiological disorder that causes extensive losses of tomato production in fields or greenhouses. Blossom-end rot has been long recognized as Ca-deficiency disorder (Evans and Troxler, 1953; Geraldson, 1957; Maynard et al., 1957), which may be induced by drought (Stout, 1934), salinity (Robbins, 1937), K fertilization (Raleigh and Chukka, 1944), NH_4^+ fertilization (Spencer and Beckenbach, 1949), and low Ca supply (Lyon et al., 1942). The form of N fertilization in relation to BER has been investigated considerably because of the strong restrictive effects that NH_4^+ has on cation accumulation by plants (Barker et al., 1966; Pill et al., 1978; Wilcox et al., 1973).

In agreement with other researchers (Pill and Lambeth, 1980; Pill et al., 1978; Wilcox et al., 1973), we have noted a high incidence of BER on tomatoes grown with $\text{NH}_4\text{-N}$ and have observed also that BER-affected fruit ripen more rapidly than normal fruit (unpublished data). A significant correlation between incidence of BER and uniform ripening was noted among some tomato genotypes (Trinklein and Lambeth, 1976). The phenomenon of early ripening of BER-affected fruit may be controlled endogenously by Ca levels (Rigney and Wills, 1981; Wills et al., 1977) and ethylene production in the fruit (Hoffman and Yang, 1980). Ammonium nutrition suppresses Ca accumulation by plants (Greenleaf and Adams, 1969; Pill et al., 1978), perhaps resulting in restricted Ca levels in fruit and advanced ripening (Rigney and Wills, 1981; Wills et al., 1977). Ethylene evolution by tomato foliage is enhanced markedly by NH_4^+ toxicity (Corey et al., 1987). Perhaps the stress of NH_4^+ toxicity also enhances ethylene biosynthesis by fruit. The present research investigated the relationships among $\text{NH}_4\text{-N}$ nutrition, Ca accumulation, and ethylene production by fruit that were damaged by BER.

Materials and Methods

This research was conducted in the spring and summer seasons in the greenhouse and field in 1990. In one experiment started in April in the greenhouse, 'Heinz 1350' tomato plants were grown

in a soil-based medium [7 sandy loam : 3 peatmoss : 2 sand (by volume) with 3 g limestone and 3 g ordinary superphosphate, 0N–8.8P–0K (per liter)] in 8.5-liter plastic nursery pots (21.5 × 21.5 cm). Ten plants in each of two nutritional regimes were grown in a completely random design. One regime provided N nutrition from daily applications of 100 ml 0.02 M $(\text{NH}_4)_2\text{SO}_4$ to impose NH_4^+ toxicity, as indicated by the development of BER and stem lesions (Maynard et al., 1966). Plants in the other regime were fertilized biweekly with 400 ml 20N–8.8P–16.6K fertilizer (urea, ammonium phosphate, and potassium nitrate) supplying 200 mg N/liter; 26% of the initial supply of N from this fertilizer was as NO_3^- . Because of the inclusion of NO_3^- in the fertilizer and likelihood of nitrification in the medium, the plants were identified as $\text{NO}_3\text{-grown}$ plants. The latter regime produced plants with no toxicity or deficiency symptoms and no BER. The fruit harvested from this experiment were used to compare ethylene evolution and $\text{NH}_4\text{-N}$ accumulation by normal and BER-affected fruit from $\text{NH}_4\text{-stressed}$ plants and normal fruit from unstressed ($\text{NO}_3\text{-grown}$) plants. Green fruit were used in these comparisons. Preclimatic fruit such as these generally have low ethylene evolution rates (Hoffman and Yang, 1980).

In another experiment started in March, 'Vendor' tomato plants were grown in a peatmoss–vermiculite–perlite medium (peat-lite) and fertilized with the same urea, ammonium phosphate, and potassium nitrate fertilizer. None of the 10 plants in this group showed any foliar toxicity or deficiency symptoms, but BER frequency was high among the fruit. After ethylene evolution was measured in fruit from this group of plants, half of the plants was fertilized with $\text{Ca}(\text{NO}_3)_2$ to provide 1 g Ca/pot (8.5-liter containers). Two weeks after fertilization, fruit were taken to determine Ca concentration.

In the field experiment started in May in a Hadley fine sandy loam at South Deerfield, Mass., 'Heinz 1350' plants were fertilized only with 80 kg N/ha from NH_4NO_3 applied before planting. None of these plants showed foliar toxicity or deficiency symptoms, and none produced fruit with BER. Fruit from this experiment were used to determine ethylene evolution and $\text{NH}_4\text{-N}$ accumulation at different stages of ripening: green, 100% green; breaker, <10% pink or red; turning, 10% to 30% tannish yellow, pink, or red; ripe, >60% pink or red. These groupings were used because there were no differences in ethylene evolution between immature green or mature green fruit or among fruit ripened past the turning stage.

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In all experiments, ethylene was measured with whole fruit sealed in wide-mouth canning jars fitted with rubber septa in screw-top lids. Portions of the atmosphere in the jars were removed with a syringe to analyze ethylene evolution by gas chromatography (Corey et al., 1987). After ethylene evolution was determined, fruit were sectioned radially, and 10-g portions were saved for $\text{NH}_4\text{-N}$ analysis. The remaining portions of the fruit were weighed and dried to determine dry weights for calculating $\text{NH}_4\text{-N}$ on a dry-weight basis and for elemental analyses. Ammonium-N was determined volumetrically (Barker and Volk, 1964) on extracts made by homogenizing the 10 g of fruit in 50 ml of 1 M KCl + 0.02 M CuSO_4 and vacuum-filtering the homogenate. Fruit pH was determined electrometrically with a glass electrode and saturated calomel standard electrode on fruit juice extracted by mashing the fruit with a mortar and pestle and vacuum-filtering the resulting pulp. Calcium, Mg, and K concentrations were determined by atomic absorption spectrophotometry on 1 M HCl extracts of the dried fruit samples (Sahrawat, 1987). All determinations were made on at least duplicate samples of fruit taken on a given day, and replications were provided by repeating these determinations with new fruit on different days. Data were processed by analysis of variance (Steele and Torrie, 1980).

Results

Ethylene evolution and ammonium accumulation by fruit. Ethylene evolution and $\text{NH}_4\text{-N}$ accumulation are reported for green fruit with or without BER from plants receiving $\text{NH}_4\text{-N}$ nutrition and by unblemished fruit of 'Heinz 1350' plants receiving $\text{NO}_3\text{-N}$ nutrition (Fig. 1). Fruit with BER had the highest rates of ethylene evolution of the group. Fruit without BER from plants receiving $\text{NH}_4\text{-N}$ nutrition had much lower ethylene evolution but did not have $\text{NH}_4\text{-N}$ concentrations different from those of the fruit showing BER. On one occasion, $\text{NH}_4\text{-N}$ -grown fruit without BER at harvest developed BER after 24 h of storage at room temperature. None of the fruit from plants receiving $\text{NO}_3\text{-N}$ nutrition had BER, and all had low ethylene evolution rates and $\text{NH}_4\text{-N}$ accumulation

relative to the fruit from plants receiving $\text{NH}_4\text{-N}$ nutrition.

'Vendor' tomatoes grown in peat-lite had high incidences of BER at the green, breaker, turning, and ripe development stages. Ethylene evolution by these fruit rose with increasing ripening stage, but there were no differences in ethylene evolution between fruit with and without BER (Fig. 2). Ammonium-N accumulation did not vary with BER condition of fruit, averaging 0.14 mg N/g fresh weight in fruit with BER and 0.18 mg N/g fresh weight in fruit without BER. These concentrations of $\text{NH}_4\text{-N}$ are about half of those occurring in fruit of plants grown with $\text{NH}_4\text{-N}$ (Fig. 1).

Ethylene evolution by 'Heinz 1350' tomatoes grown in the field with $\text{NO}_3\text{-N}$ increased with advancing ripening stage (Fig. 3). Ammonium-N concentrations did not vary with ripening stage, remaining at ≈ 0.15 mg N/g fresh weight, essentially the same as those in 'Vendor' tomatoes.

Calcium in fruit. Calcium concentrations were higher in 'Heinz 1350' fruit receiving $\text{NO}_3\text{-N}$ nutrition than in fruit of plants receiving $\text{NH}_4\text{-N}$ nutrition (Table 1). Calcium did not differ with respect to BER incidence on the $\text{NH}_4\text{-N}$ -grown fruit. Concentrations of Mg or K did not vary with fruit condition or nutritional status (Table 1). With 'Vendor', Ca concentrations differed slightly but significantly whether the fruit had BER or not (Table 2). Fertilization with Ca increased fruit Ca, with measurements taken 2.5 weeks after Ca fertilizer was applied. Blossom-end rot at this time was confined largely to ripe fruit, with very few green fruit having BER, a result indicating that fertilization had arrested BER development. Ripe fruit had higher Ca than green fruit, with the increases in fruit Ca being restricted to fruit from fertilized plants. Neither K nor Mg varied with BER status, with an average K concentration of 2.57% and an average Mg concentration of 0.16% on a dry-weight basis.

Fruit pH. The juice extracted from green 'Heinz 1350' fruit with $\text{NH}_4\text{-N}$ -induced BER was pH 5.5, which was higher than that (pH 4.5) from fruit of $\text{NO}_3\text{-N}$ -grown plants. Extracts from $\text{NH}_4\text{-N}$ -grown fruit without BER were pH 5.4. For ripening fruit with $\text{NH}_4\text{-N}$ -induced BER, juice pH was 4.8. Juice from ripening $\text{NO}_3\text{-N}$ -grown fruit was pH 4.4.

Fig. 1. Ethylene evolution and $\text{NH}_4\text{-N}$ concentrations in green 'Heinz 1350' tomatoes with $\text{NH}_4\text{-N}$ with or without blossom-end rot (BER), and with $\text{NO}_3\text{-N}$. Letters over bars indicate significant difference by Duncan's multiple range test among means for ethylene evolution or $\text{NH}_4\text{-N}$ concentration.

Fig. 2. Ethylene evolution of 'Vendor' tomatoes at different ripeness stages with or without blossom-end rot (BER). Results of analysis of variance: BER, nonsignificant; ripeness, significant ($P \leq 0.01$); BER $\times$ ripeness, nonsignificant.

Fig. 3. Ethylene evolution and NH₄-N concentrations in 'Heinz 1350' tomatoes at different ripeness stages. Results of analysis of variance: ethylene, significant increase with ripening ($P \leq 0.01$); NH₄-N, no change with ripening.

Table 1. Elemental composition of 'Heinz 1350' tomatoes with or without blossom-end rot (BER) from plants grown on NH₄-N or NO₃-N nutrition.

Condition of fruit		Concn (mg·kg ⁻¹ dry wt)		
N source	BER ^z	Ca	Mg	K
NH ₄	BER	390 a	1141 a	26,062 a
NH ₄	No BER	382 a	1106 a	25,280 a
NO ₃	No BER	682 b	1209 a	25,502 a

^zCondition at harvest. None of the NO₃-N-grown tomatoes had BER. Mean separation within columns by LSD at $P \leq 0.01$.

Discussion

Plants stressed by NH₄-N nutrition produced fruit with high incidences of BER. These fruit accumulated higher NH₄-N concentrations, had higher ethylene evolution rates when green, and had lower Ca concentrations than unblemished fruit from plants receiving NO₃-N nutrition. The accumulation of NH₄-N was a unique characteristic of fruit from plants grown on NH₄-N, for neither normally ripening fruit nor BER fruit of plants receiving NO₃-N showed enhanced NH₄-N levels. Stresses such as NH₄⁺ toxicity (Corey et al., 1987), certain nutrient deficiencies (Feng

Table 2. Concentrations of Ca in ripe or green 'Vendor' tomatoes with or without blossom-end rot (BER) and before or after Ca fertilization.

Condition of fruit and means	Ca concn (mg·kg ⁻¹ dry wt)				
	Before fertilization		After fertilization		Mean
	Green	Ripe	Green	Ripe	
BER	625	563	1075	1503	957
No BER	694	669	1162	1663	1089*
Mean (ripeness × fertilization)	660 a ²	616 a	1118 b	1583 c	
Mean (fertilization)	669		1377**		

²Mean separation within the ripeness × fertilization interaction by LSD at $P \leq 0.01$. BER condition × fertilization × ripeness interaction was nonsignificant.

*,**Significant by F test at $P \leq 0.05$ or 0.01 , respectively.

and Barker, 1992a), and salinity (Feng and Barker, 1992b) lead to $\text{NH}_4\text{-N}$ accumulation in tomato foliage. Ammonium-N accumulation seems to be followed by ethylene evolution (Barker and Corey, 1991; Feng and Barker, 1993). With plants grown with $\text{NH}_4\text{-N}$, we noted that $\text{NH}_4\text{-N}$ accumulated in fruit without BER and that ethylene evolution was lower from these fruit than from those with BER. These observations and BER development during storage of harvested, green, $\text{NH}_4\text{-N}$ -grown fruit suggest that $\text{NH}_4\text{-N}$ accumulation precedes BER development and ethylene biosynthesis. Perhaps, in parallel with the physiology of foliage, $\text{NH}_4\text{-N}$ accumulation in green fruit initiates injury and processes that lead to ethylene evolution (Barker and Corey, 1991).

The finding of lower total Ca concentrations in $\text{NH}_4\text{-N}$ -grown fruit agrees with results in which $\text{NH}_4\text{-N}$ nutrition or depressed Ca levels were related to enhanced BER incidence (Greenleaf and Adams, 1969; Pill et al., 1978). Fruit with BER from plants grown with $\text{NH}_4\text{-N}$ had about half the Ca concentration of unblemished fruit from plants grown with $\text{NO}_3\text{-N}$. With plants on $\text{NO}_3\text{-N}$ nutrition, differences in fruit Ca concentrations were small but significant between BER and normal fruit. Although it is understood clearly (Geraldson, 1957; Maynard et al., 1957) that BER is a result of Ca deficiency, attempts to demonstrate that fact through fruit analyses have not always been successful (Murray et al., 1972; Pill and Lambeth, 1980). Differences in Ca distribution (Pill and Lambeth, 1980) or relationships of Ca with K and Mg in fruit (Murray et al., 1972) have been offered as explanations for the lack of correlation between BER and total fruit Ca. We did not measure Ca partitioning in fruit, but observed no special relationships among Ca, K, and Mg in fruit with BER, regardless of source of N nutrition.

Some similarities between the physiological responses of $\text{NH}_4\text{-N}$ -stressed fruit and bruised or diseased fruit are apparent. Bruised mature-green fruit ripened quickly and had higher ethylene evolution rates and lower titratable acidity than unblemished fruit (MacLeod et al., 1976). Ripe fruit infected with *Alternaria tenuis* or anthracnose (*Colletotrichum coccodes*) had higher pH and lower titratable acidity than uninfected fruit (Sapers et al., 1978). Ammonium accumulation was not measured in the previous studies; however, NH_4^+ has been shown to accumulate in disease-infected leaves and to be a causal agent in the injury resulting from the infections (Bashan et al., 1980; Lovrekovich et al., 1970). The deleterious effects of bruising and disease infection may have been due to NH_4^+ accumulation induced by these injuries.

The increased ethylene evolution rates and apparently increased ripening rates by green fruit from plants grown with $\text{NH}_4\text{-N}$ seem to be the result of stresses imparted by $\text{NH}_4\text{-N}$ accumulation in the fruit. Our results show that $\text{NH}_4\text{-N}$ accumulation was confined to $\text{NH}_4\text{-N}$ -grown plants, was not associated with

normal fruit ripening, and did not occur in fruit with BER induced by factors other than $\text{NH}_4\text{-N}$ nutrition. The results also suggest that restricted Ca accumulation by fruit of plants grown with $\text{NH}_4\text{-N}$ was associated with BER development.

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