Mechanism of the Development of a Calcium-Related Disorder (Bitter Pit) in Apple

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Since Delong (1936)[4] showed a close relationship between bitter pit and calcium deficiency of apple, bitter pit has been so far considered as a calcium-related physiological disorder (see Shear 1975,[16] and Bangerth 1979[2] for reviews). In addition, an excess of nitrogen, potassium or magnesium stimulates the incidence of bitter pit.[8] To understand the physiological basis of bitter pit disorder it is necessary to examine the mechanism of calcium regulation and the effect of the other elements on calcium metabolism in the fruit.

The important role of calmodulin in a number of calcium-related metabolism in plants is now clearly recognized as well as in animals,[5] suggesting that the relationship between calmodulin and bitter pit should be examined.

In the present paper we describe the induction of bitter pit by applying ammonium salt in culture solution, and the role of ammonium, calcium and calmodulin in the development of bitter pit in apple. In addition, a possible mechanism for the occurrence of bitter pit will be discussed.

Induction of bitter pit by applying ammonium salt

Each of 5-year-old trees (*Malus pumila* Mill. var. *domestica* Fuji) on *Malus prunifolia* rootstock was transferred to a culture solution (300 *l*/pot) and treated with either of ammonium or nitrate solution with or without calcium salt.[7] The pH was adjusted to 6.0–6.5 about every 10 days with 1.5 M H_2SO_4 or 3 M NaOH.

The nutrient solutions were completely renewed every month from April to November, while only in November nitrate was used instead of ammonium as the nitrogen source. The incidence of bitter pit was expressed as the percentage of affected fruit for the total number of fruit per tree.

The supply of ammonium-nitrogen led to the increase in the incidence of bitter pit as compared to that of nitrate-nitrogen (Table 1). The suppression of calcium supply facilitated the development of

Table 1. Effect of calcium concentration and nitrogen-form applied on the incidence of bitter pit at harvest time in 'Fuji' apple trees on *Malus prunifolia* rootstock[7]

Treatment	Incidence of bitter pit (%)		Yield (kg/tree)		Mean fruit weight (g)	
	1980	1981	1980	1981	1980	1981
Ammonium−calcium	79.8±6.9	64.0±18.3	17±2	21± 5	190	154
Ammonium+calcium	25.8±1.7	55.0±1.2	19±3	26±14	185	155
Nitrate − calcium	3.0±0.7	6.4±4.4	22±2	49± 5	215	185
Nitrate + calcium	0.0±0.0	0.0±0.0	24±5	41±17	209	179

Table 2. Effect of calcium and nitrogen-form applied on mineral contents (% dry weight) of healthy and affected fruit at harvest time in 1981[7]

Treatment	Nitrogen			Calcium ($\times 10^{-2}$)			Potassium			Magnesium		
	A	B	C	A	B	C	A	B	C	A	B	C
(Healthy fruit)												
Ammonium−Ca^{2+}	0.18	0.18	0.43	0.72	0.99	3.10	0.44	0.46	0.73	0.023	0.021	0.042
Ammonium+Ca^{2+}	0.22	0.21	0.49	0.72	1.10	2.99	0.47	0.44	0.73	0.023	0.020	0.041
Nitrate−Ca^{2+}	0.16	0.18	0.41	0.75	1.15	3.14	0.42	0.44	0.81	0.022	0.021	0.043
Nitrate+Ca^{2+}	0.14	0.17	0.37	1.22	1.77	5.37	0.35	0.42	0.67	0.019	0.019	0.036
(Affected fruit)												
Ammonium−Ca^{2+}	0.25	—*	—	0.52	—	—	0.40	—	—	0.019	—	—
Ammonium+Ca^{2+}	0.28	—	—	0.53	—	—	0.44	—	—	0.022	—	—

A,B and C indicate the flesh near the calyx end, the flesh near the stalk end and the core, respectively.
* Data not shown.

Table 3. Effect of calcium concentration and nitrogen-form applied on the incidence of bitter pit at harvest time in 'Fuji' apple trees on M9 rootstocks in 1981

Treatment	Incidence of bitter pit (%)	Yield (kg/tree)	Mean fruit weight (g)
Ammonium−calcium	1.8±2.5	3.0±1.0	81
Ammonium+calcium	1.0±1.4	6.9±2.6	168
Nitrate − calcium	1.6±2.2	16.3±8.5	225
Nitrate + calcium	0.0±0.0	14.1±5.0	247

The results represent the mean ±SD from 2 replicate trees.

bitter pit. The development of bitter pit significantly decreased the calcium content of and increased the nitrogen content of the fruit especially near the calyx end (Table 2).[7] The ammonium-nitrogen content of the affected fruit was also higher than that of the healthy fruit.[7]

When Fuji/M9 trees were treated with ammonium-nitrogen and low calcium, the mean fruit weight was extremely decreased although the incidence of bitter pit was kept lower than that in the case of Fuji/*Malus prunifolia* rootstock trees (Table 3), and consequently they died after the three-year-treatment. When M9 rootstock was used, the incidence of bitter pit was lower than that in the case of *Malus prunifolia* rootstock in each of the treatments. This may reflect different susceptibility to ammonium and calcium nutrition between these two rootstocks.

These indicate that bitter pit disorder can be induced by applying ammonium salt and may be associated with the level of calcium and nitrogen in the fruit.

Calcium transport in apple fruit

Bitter pit disorder tends to develop in the flesh near the calyx end beneath the peel. Calcium content is known to be very different among the portions of a fruit taken. The core has the highest content of calcium among the core, the flesh near the calyx end and the flesh near the stalk end, while the flesh near the calyx end has the lowest one. When calcium transported through the pedicel moves into the flesh near the calyx end, some of the calcium could be absorbed by various cell components, that is, mitochondria, vacuoles and so on, before arriving at the site. Lowering calcium transport into the flesh near the calyx end may lead to the increase in the incidence of bitter pit. Fig. 1 shows an example of lowering the calcium transport.[7] Apple fruit, which should be affected by bitter pit by the harvest time through the ammonium supply, have a higher mitochondrial calcium uptake activity than those of the control (nitrate applied). In fact, the ratios of the

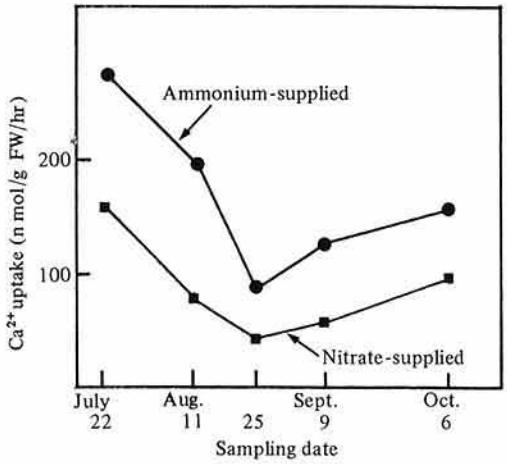

Fig. 1. Effect of nitrogen-form applied on the mitochondrial calcium uptake of apple fruit[7]

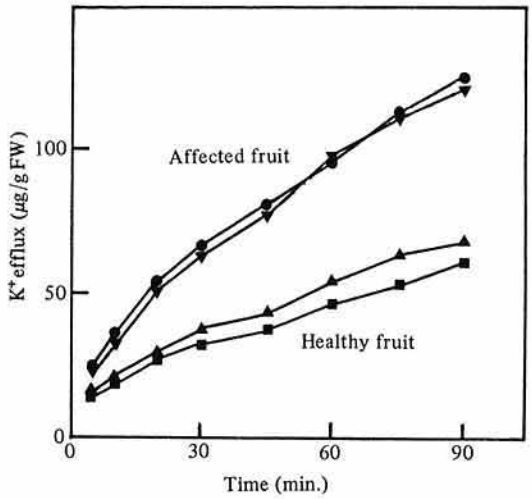

Fig. 2. Changes in potassium efflux from the fruit flesh by the development of bitter pit in apple

calcium content in the flesh near the calyx end to that of the core and of the flesh near the stalk end were lower in the affected fruit than in the healthy fruit.[8] The supply of ammonium may restrict the calcium transport to the flesh near the calyx end through the stimulation of the mitochondrial activity.

Changes in membrane permeability and the distribution of calcium by the development of bitter pit in apple fruit

Van Goor (1971)[17] reported that the ion permeability of apple fruit tissue diminished as a result of calcium sprays, demonstrating that an increase in membrane permeability may be involved in the development of bitter pit. Therefore, the membrane permeability of the fruit was examined by determining potassium efflux from the tissues. As Fig. 2 shows, the tissues taken from the affected fruit had a higher potassium efflux than those from the healthy fruit. The affected fruit seems to have a sort of leaky membranes.

Calcium accumulation has been observed in the pitted area by several researchers.[3,12] Ford (1979)[6] showed that calcium accumulated in the pitted area after bitter pit had happened. So we investigated calcium distribution in the tissues near the pitted area by using an X-ray microprobe. We also found calcium accumulation in the pitted area. However, in some cases calcium accumulation occurs in the boundary layer between sound and pitted areas, but not in the pitted area (Plate 1). These findings support the result of Ford (1979) stated above.

Induction of bitter pit-like spots by calmodulin antagonist infiltration

Some calmodulin antagonists, fluphenazine, chlorpromazine, W-7* and W-5**, were used in this experiment. Each of them at 0.1 mM was infiltrated into each fruit for 20 min under reduced pressure (0.1–0.2 atm) at room temperature. After the treatment the fruit were left at 20 °C under humid condition. The effects of the infiltration of the calmodulin antagonists into the fruit on the occurrence of bitter pit-like spots were examined.[7,9] Numerous bitter pit-like spots were observed in the fruit treated with fluphenazine, chlorpromazine or W-7, much less so by W-5 which is an analogue of W-7 and much less effective as a calmodulin antagonist (Table 4).

* W-7: N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide

** W-5: N-(6-aminohexyl)-1-naphthalenesulfonamide

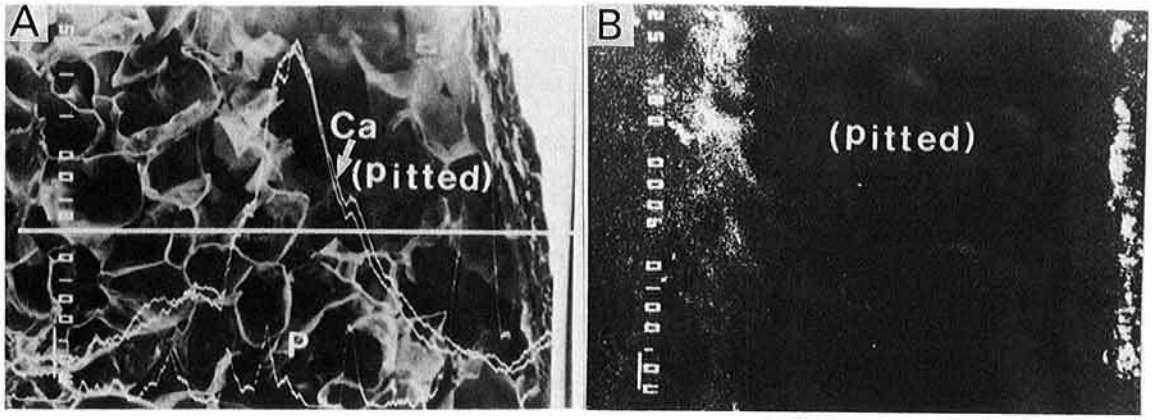

Plate 1. Calcium distribution in apple fruit affected by bitter pit

A: Line scan profile for Ca across bitter pit of apple fruit
B: X-ray distribution image for Ca at the pitted area of apple fruit
The bars represent 100 μm.

Table 4. Induction of bitter pit-like spots by calmodulin antagonist infiltration

Treatment	Number of spot
Water	0 ± 0
W-5	8 ± 5
W-7	148 ± 13
Chlorpromazine (0.05 mM)	64 ± 33
Chlorpromazine (0.1 mM)	309±146
	(Index)
Water	−
Chlorpromazine (0.1 mM)	+++
Fluphenazine (0.1 mM)	++++

Values are means±SD (n=2).[7,9]

These findings indicate that calmodulin inactivation in apple fruit may be involved in the development of bitter pit.

Role of calcium and calmodulin in the development of bitter pit

To maintain the normal metabolism of plant cell it is necessary to keep the concentration of calcium low in the cytoplasm through various ATP-dependent calcium transport systems of the cell organs, some of which are calmodulin-dependent.[5,10] Recently, it has been shown that apple fruit had low molecular weight calmodulin inhibitors, that is, flavonoids,[14,15] which are mainly located in the vacuoles.[18] We also found that the flavonoid fraction extracted from apple fruit inhibited calmodulin-dependent phosphodiesterase activity (data not shown). When these inhibitors were leaked into the cytoplasm, calmodulin-dependent calcium transport systems would be inhibited and then the cell be decomposed. This may be a reason why apple tissues affected by bitter pit were destroyed (Plate 1).

Marinos (1962)[11] showed by electromicroscopic observation of barley tissues that calcium is essential for maintaining structural integrity of various membranes of the cells. Bangerth (1973)[1] noted that bitter pit was associated with the deterioration of plasma membranes and tonoplasts. Tazawa et al. (1976) reported that perfusion of the cell sap with buffer containing no calcium or containing EGTA breaks the vacuolar membrane (cited by Ohsumi and Anraku 1983).[13] These indicate that calcium deficiency may lead to the decomposition of membrane structures in plant cell. If the level of calcium in apple fruit is kept quite low, the integrity of tonoplasts and plasma membranes in the site of the cell could be lowered, then it would be followed by the development of bitter pit.

The role of calcium-regulation in maintaining these membrane structures should be examined to elucidate the mechanism of the development of bitter pit.

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