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The Role of Calcium and Cell Permeability in the Disease Blossom-end Rot of Tomatoes

By

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Abstract

Research was carried out concerning the cell-physiological backgrounds of the disease blossom-end rot of tomatoes, which, like bitter pit of apple and tipburn of lettuce, is a Ca-deficiency disease. In our experiments tomatoes with the least amounts of Ca were attacked by the disease. We estimated the ion permeability of tissues with different Ca levels.

Permeability was found to increase with decreasing Ca contents in the tissue. The increase accelerated with the beginning of the disease. The artificial rise in the permeability caused by ether and decenylsuccinic acid resulted in an increased occurrence of the disease.

It is probable that the Ca-deficiency causes the necrosis of the tissue in blossom-end rot and that the shortage of calcium among others affects the disorganization of membranes and organelles in the cell.

Introduction

In horticultural practice several diseases have been detected which are caused by a local deficiency of a certain element in a part of a plant. In other words the optimum ratio between the different ions is not attained. In this introduction special attention will be given to those diseases, which appear when there is insufficient Ca in a part of a plant in relation to the potassium concentration.

Bitter pit is a disease of apples which in general is attributed to a local deficiency of Ca (Garman and Matthis 1956, Van Schreven *et al.* 1962, Van der Boon *et al.* 1962, Schahrestany 1964, Kepka 1964). Tepe (1965), however, explains it as a result of the oxidation of the tissue content. Possibly the contradictions which appear in the analytical data can be explained

from the fact that in some experiments estimations have been performed on dead regions of the fruits. Tipburn of cabbage and lettuce — withered edges and spots — is also an illness which arises with low calcium content (Maynard *et al.* 1965).

In tomatoes a local low content of Ca in the apex can cause a necrosis of the tip tissue (Maynard *et al.* 1957, Sonneveld 1963, Bruins 1965, El-Asdoudi 1966, Wiersum 1966).

Fertilizing with Ca causes less incidence of this disease, which is called blossom-end rot (Riggleman 1964). The first sign of the disease is a darker green colour of the apex of unripe tomatoes, which usually have a diameter of 1-3 cm. This change of colour seems to be an effect of an infiltration of the intercellulars with tissue moisture. Afterwards the apex grows brown and indent, and many cells of this part of the fruit have probably already died. We have carried out research on such alterations in the cell which appear when there is insufficient Ca in the tissue. The investigations on the cytology and cell physiology of the illness are very few.

Anatomical work demonstrates certain alterations in the cytoplasm. There appear incrustations, probably with a protein character, in the protoplasm. But it is not clear whether the alterations of the plasma or the disintegration of the cell-wall are primary (Brooks 1914, Spurr 1959).

As a result of experiments with young barley roots Marschner and Günther (1964) found that with insufficient Ca the formation of tonoplast and vacuole is blocked. They also observed a larger K efflux and Na uptake in a NaCl solution. The cells with a low calcium content can only accumulate a low concentration of ions. Also Marinos (1962), who investigated Ca-poor tissues electron-microscopically, found a destruction of plasmalemma and organells. He worked with barley sprouts. All these experiments have been done with young cells in which the vacuole is developing. In experiments with the algae *Hydrodictyon* and *Sphaeroplea* Frank (1962) obtained, in cases of Ca deficiency, a sudden breakdown of the tonoplast of the cells. Van Steveninck (1965) found also that the permeability for K is much higher in the root cells of beets when 75 per cent of the Ca has been removed. With more calcium in the root segments of corn Handley *et al.* (1965) obtained a decreased non-metabolic uptake owing to a lower permeability of the outer membrane. Foote and Hanson (1964) treated root pieces of soyabean with K EDTA to bind Ca from the tissue. This caused initially an increase in the accumulated quantity of K; when 65 per cent of the Ca content had disappeared however, K leached out of the tissue. In general it appears that insufficient Ca in the cell gives disintegration of membranes, *e.g.* plasmalemma and tonoplast, and a higher permeability for ions, which gives a higher loss of ions.

Also when plant tissues become senescent one gets comparable phenomena. Eilam (1965) *e.g.* found, with cotyledons of *Phaseolus multiflorus*, an increase in "apparent free space", the permeability for K, and a decrease in respiration. Thus there are reasons for interpreting data about permeability with caution.

Possibly there is also a relation between the above-mentioned phenomena and plant diseases caused by organisms of the tribe *Fusarium*. The symptoms that appear are rather similar. It is possible that the fusaric acid produced

by these fungi binds some Ca from the tissue. The permeability for water becomes higher when the tissues are treated with fusaric acid in concentrations of 10^{-6} M (Heinrich 1962).

About the structure of the membranes we are informed by Frey-Wyssling and Mühlethaler (1965, pp. 140-161). It is supposed that they consist of lipid layers coated with proteins. Also a globular structure has been proposed, which presumes pore filtration as one of the possible explanations for permeability. The barriers for the permeation are plasmalemma and tonoplast; the mesoplasm would have a larger permeability for ions. Levitt (1960) defends the membrane theory on account of the low surface tension of the protoplast and the high electrical resistance of the cell surface. Strong support comes from electron-microscopical work.

The purpose of the experiments described in this paper is to investigate the relation between the induction of blossom-end rot, the Ca level and the permeability of the tissue, especially for ions. Permeability has been estimated by measuring the conductivity of the leaching fluid and its K content. A formula for the leaching of K is given.

The velocity of desiccation of the tissue is also measured. Some substances which are known to increase the permeability of the tissue are tested as to their influence on the disease, namely decenylsuccinic acid, which increases the waterpermeability according to experiments of Kuiper (1964), and diethyl-ether. Using tomato tissue we also studied the effect of these substances on the permeability for ions.

The central subject of the present study is the role of the membranes in Ca-deficiency diseases.

Materials and Methods

In general we worked with tomato fruits from plants grown in water culture. The breed Eurocross sensible to the blossom-end rot was germinated on poor sand.

Table 1 gives the solutions in which the plants were grown subsequently.

After two weeks the plants were put into 1-litre plastic pots with the culture fluid 40/50 K/Ca. The solution was renewed every week. About a month later 10-litre plastic vessels were used and the plants were grown in two solutions, one solution with low Ca (K/Ca 80/10 or 70/20), and another with high Ca (K/Ca 20/70). The solutions were refreshed every week until the plants were 30 cm high. From 30 cm until 40 cm the refreshment was made every 3 or 4 days, and after that every 2 or 3 days.

Table 1. Concentration of ions in the culture-solutions.

Equivalent K/Ca	Ion concentration (mgion/l)						
	NO ₃ ⁻	H ₂ PO ₄ ⁻	SO ₄ ²⁻	K ⁺	Ca ²⁺	Mg ²⁺	Total
80/10 Ca low	10.62	0.88	3.09	12.76	0.80	1.79	29.94
70/20 Ca low	10.92	0.91	3.18	11.50	1.64	1.85	30.00
40/50	11.82	0.98	2.62	7.18	4.48	2.02	29.10
20/70 Ca high	12.58	1.05	3.68	3.82	6.70	2.14	29.97

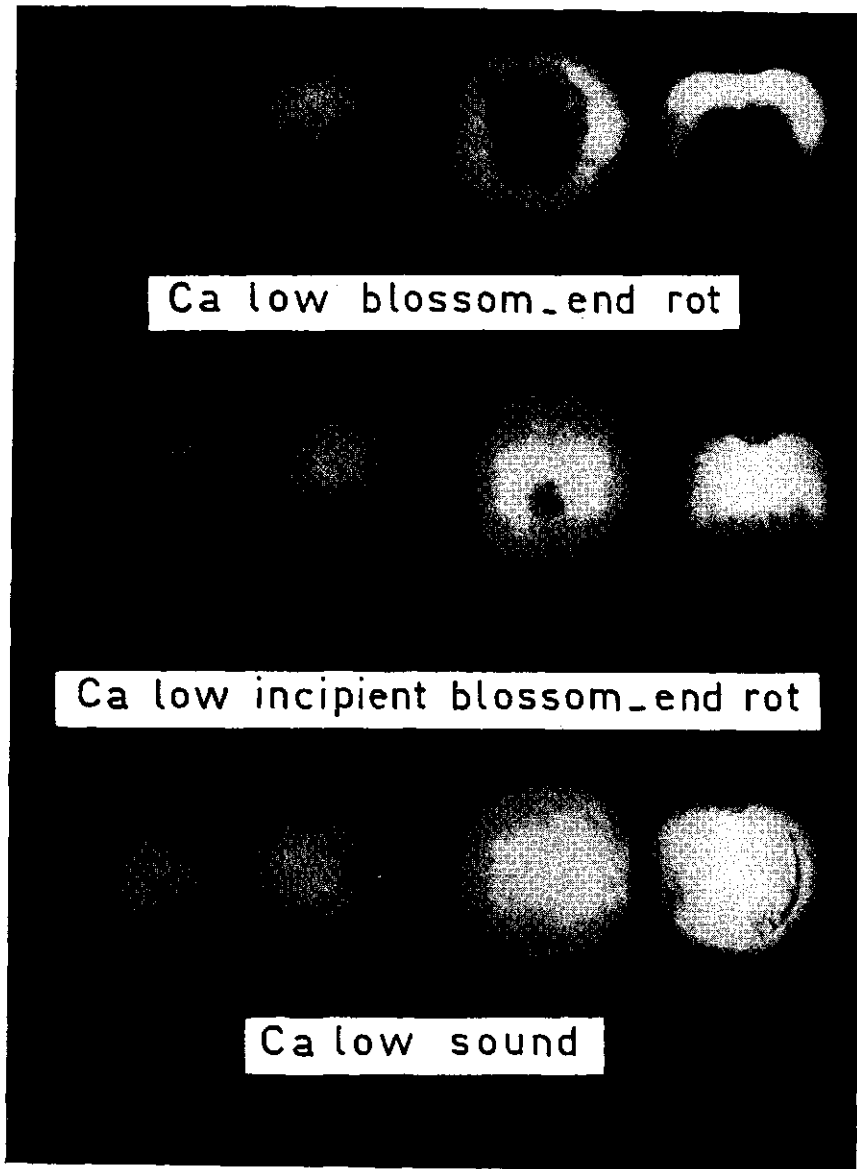


Figure 1. Picture of tomatoes with and without blossom-end rot.

Information concerning the solutions from Table 1 was given by A. A. Steiner (private communication). They all have the same osmotic value and contain among the microelements especially B, Zn, Cu, Mn, Mo, and Fe.

In all experiments on permeability we harvested three series of tomatoes, namely fruits from the solution with much Ca, fruits which were apparently intact, and

fruits with the first signs of blossom-end rot from the low Ca-solution. The beginning of blossom-end rot presents itself by a dark-green colouring of the tip of the tomatoes. In the tomatoes of the latter series we observed although seldom brown pieces of tissue, which certainly contain some dead cells. From every series we selected tomatoes of the same dimensions. Figure 1 shows comparable tomatoes with and without beginning blossom-end rot and further a case of a heavy attack of the disease. Generally we worked with tomatoes of 1-2 cm diameter.

From the fruits we cut off the apex (about one third part of the tomato) in a comparable way for the three series. In the experiments we used only fruit wall and partitions; the other fruit parts were removed. The apex part was cut in two parts, one fourth for estimating K and Ca contents, the remaining part for measurement of permeability. The tissue for analysis was dried and ashed, after which K was estimated flame-spectrophotometrically and Ca oxidimetrically with potassium permanganate.

The tissue for estimation of permeability was washed three times during 60, 60 and 10 s in distilled water. It was dried on filter paper between each washing. The weight was estimated, and after that the tissue was dropped into a beaker with 50 ml distilled water. In the beaker the specific resistance was measured after 1.5, 3 and 4.5 hours. From this value the increase in conductivity was calculated.

After 4.5 hours a sample was taken for estimation of the K concentration in the solution, from which the percentage of permeated K was determined, related to the initial quantity present. Further we tried to calculate a constant for the permeability which we thought to be independent of the concentration.

It was based on the diffusion equation:

$$dC_t = kA (C_t - c_t) dt \quad (1)$$

After integration we got:

$$\log \left[\left(\frac{V}{5 \cdot 10^{-5} + V} \cdot C_0 \right) - c_t \right] = \log \left[\frac{V}{5 \cdot 10^{-5} + V} \cdot C_0 \right] - \left[k \cdot A \cdot \frac{V + 5 \cdot 10^{-5}}{V} \cdot t \right] \quad (2)$$

In these equations the meaning of the symbols is the following:

- c_t = concentration in water on time t in mgion K^+/l ,
- C_0, C_t = concentration in tissue on time 0 and time t in mgion K^+/l cell sap,
- V = tissue volume in m^3 ,
- A = tissue-surface in m^2 ,
- t = time in hours,
- k = permeation constant in $hour^{-1} m^{-2}$.

Since the tissue surface is unknown however, we have calculated a constant per gram, which is allowed when one compares three approximately equally heavy portions of tissue. The velocity with which the tissue loses water was estimated from small pieces of the apex of tomatoes on a watch-glass on dry $CaCl_2$ in an exsiccator. The decrease in weight was calculated as the percentage of moisture that has been lost. The ether treatment was made with tomato plants in 5-litre plastic pots containing a sand-peat mixture with 6 g CaO and 1.7 g K per pot. They were manured during the experiment with K_2SO_4 and NH_4NO_3 (about 0.8 g K and 0.7 g N).

The first and second trusses were employed for the treatments. The trusses were

bound in plastic bags of about $\frac{1}{2}$ l volume with 1 ml ether on a piece of wadding. The ether treatments were applied one or twice a day during a quarter of an hour.

Every morning and evening the percentage blossom-end rot was counted. With a similar series of plants experiments on the influence of decenylsuccinic acid were run. Twice a day the fruits were rubbed in with a 10^{-2} M solution of decenylsuccinic acid. The control fruits were treated with distilled water acidified with HCl to the same pH. After certain periods of time the percentage of blossom-end rot was counted.

The ether and decenylsuccinic acid experiments were made in a not regulated hot-house; sometimes we raised the temperature for getting more blossom-end rot.

To test if ether and decenylsuccinic acid really increase the permeability of the tomato tissue for ions pieces of the fruit wall were treated in petri dishes with ether vapour and decenylsuccinic acid solution on filter paper during approximately 3 hours.

After washing of the tissue the permeability was measured in the way described above.

Results

Orientating experiments have been performed with tissues of tomatoes and apples, treated with oxalic acid, water, and calcium salts. They are likely to show an increasing content of calcium. The leaching of ions was measured in water. The conductivity and K content of the water were estimated. The conductivity was found to be as large as that of a KCl-solution with the same K concentration as the leaching fluid. So we probably measured mainly potassium in our conductivity experiments. A pretreatment of apple tissue

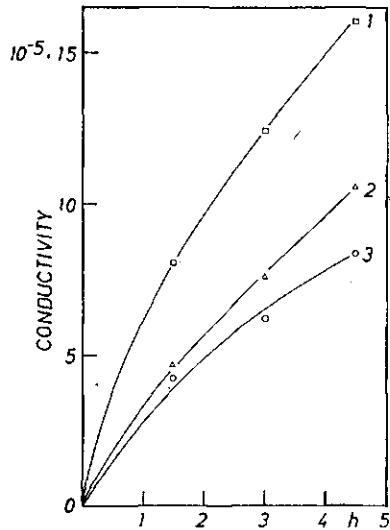


Figure 2. Increase in conductivity ($\Omega^{-1} \text{ cm}^{-1} \text{ g}^{-1}$) of distilled water by leakage from the tissue of the tomato fruit. Curves: (1) Ca low + advance of blossom-end rot; (2) Ca low; (3) Ca high.

Table 2. Constant k per g for permeability experiments of different duration.

Hours after start of experiment	Ca high	Ca low
1.5	31.6×10^{-5}	32.4×10^{-5}
3	22.0×10^{-5}	24.0×10^{-5}
4.5	14.5×10^{-5}	17.1×10^{-5}
6	11.0×10^{-5}	12.9×10^{-5}

Table 3. Some data of permeability for ions of the different tissues (about ten tomatoes of 1-2 cm).

Parameter	aq. K/Ca in culture solution		
	Ca high 20/70	Ca low 80/10 or 70/20	Ca low at beginning of disease 80/10 or 70/20
Blossom-end rot, % of total	5	34	34
Ca % on dry weight	0.091	0.043	0.031
K % on dry weight	6.56	6.90	7.31
K/Ca	72	160	237
K % permeated after 4.5 hour ...	21.1	22.4	36.1
Constant k per g	43.1×10^{-5}	46.4×10^{-5}	82×10^{-5}

with oxalic acid, distilled water and $\text{Ca}(\text{NO}_3)_2$ gave a leaching loss of 40, 30 and 25 per cent of the potassium present.

Besides it was established that immersing the tissue in solutions with a high osmotic value gives a lower conductivity which means that the diffusion of ions out of the tissue is not dependent on a water displacement.

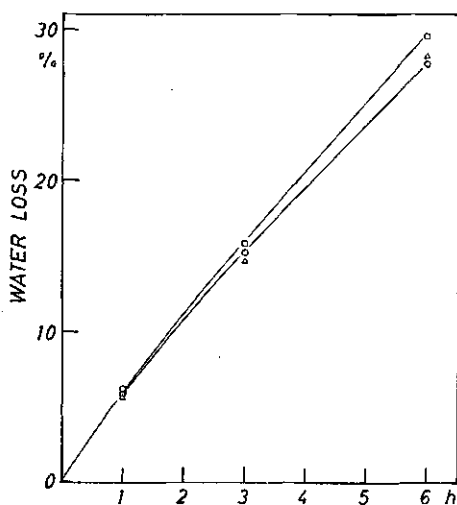


Figure 3. Percentage water loss above CaCl_2 from the tissue of the apex of the tomato fruit. (□) Ca low + advance of blossom-end rot; (Δ) Ca low; (○) Ca high.

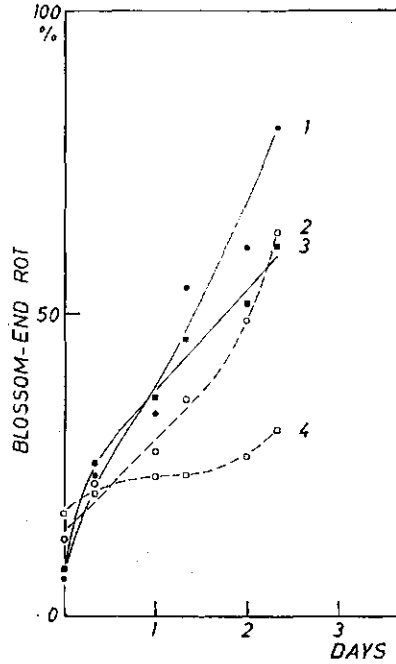


Figure 4. Increase in blossom-end rot in time, with and without ether treatment. Abscissa: days after the beginning of experiment. Curves: (1) Treated ether truss 2; (2) untreated blanc truss 2; (3) treated ether truss 1; (4) untreated blanc truss 1.

A Ca-rich tissue appeared to take up more water from the distilled water than a Ca-poor one.

In Figure 2 is shown the increase in conductivity of the fluid caused by

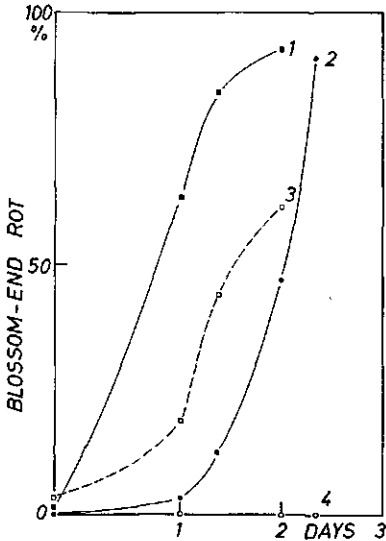


Figure 5. Increase in blossom-end rot in time with and without decenylsuccinic acid treatment. Abscissa: days after the beginning of experiment. Curves: (1) Decenylsuccinic acid truss 1; (2) the same, truss 2; (3) blanc truss 1; (4) blanc truss 2.

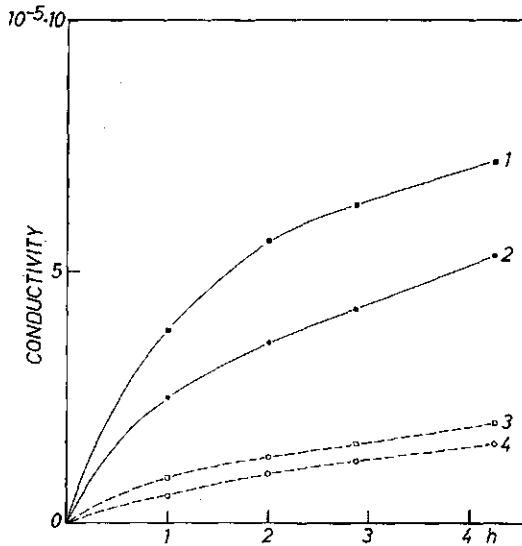


Figure 6. Increase in conductivity ($\Omega^{-1} \text{ cm}^{-1} \text{ g}^{-1}$) of distilled water by leakage from the tomato fruit tissue, with and without treatment with ether and decenylsuccinic acid. Curves: (1) Ether; (2) decenylsuccinic acid; (3) blanc ether; (4) blanc decenylsuccinic acid.

immersed tomato tissue with different Ca-levels. A low Ca content of the tissue gives more leakage of ions — especially K — from the cells; the beginning of blossom-end rot gives rise to a stronger increase in conductivity than is accounted for by the relative increase of the K content of the tissue.

Studying the permeability of the K^+ -ion in relation to the Ca status of the tissue we used two methods of calculating this K leakage, namely the percentage K, which has permeated, and the permeation constant. The permeation constant was calculated from equation 2.

Firstly we tested the equation for different periods of time. The equation is to be considered reliable when one gets nearly the same constant for different periods of time.

The result (Table 2) does not agree with equation 2. Possible explanations will be given in the discussion. Probably it is better to give the permeability for an ion as the percentage of the total quantity present which has been leached out after a standard period of time.

In Table 3 we have summarized the data about the Ca and K contents of the tip tissue and its permeability for ions.

The Ca content of the tissue is lower when blossom-end rot appears. The percentage of the disease is much higher in plants with a low Ca status. The permeability of the tissue for K^+ -ions increases with declining calcium content. The increase becomes still more rapid when the blossom-end rot begins to appear in the apex.

Figure 3 presents the mean of a number of experiments on the velocity of desiccation of the three tissues in an atmosphere dried with CaCl_2 . The differences are small; possibly the velocity is somewhat higher for the tissue with beginning symptoms of blossom-end rot.

Figure 4 shows the results of the ether treatments on the percentage of

Table 4. *Per cent K leached from tomato tissue after 4.5 hours with different treatments.*

Treatment	Exp. 1	Exp. 2	Mean
Ether	50.3	51.5	50.9
„ blanc	12.4	10.0	11.2
Decenylsuccinic acid	36.4	33.7	35.1
„ „ blanc	8.5	9.5	9.0

blossom-end rot. We see that the ether treatments increase the disease considerably. Probably the curves will coincide in a later stage when only very resistant tomatoes are left.

Decenylsuccinic acid, another substance which makes the membranes more open, increases the velocity at which tomatoes become attacked by blossom-end rot (Figure 5).

From Figure 6 and Table 4 is evident that ether as well as decenylsuccinic acid enhance the permeability for ions of tomato fruit tissue.

Discussion

The permeability for ions of pieces of the unripe fruit of the tomato has been measured in different ways. Firstly, estimations were made of the increase in conductivity of the distilled water into which the pieces had been immersed. The increase is larger for the Ca-poor tissue and especially for that of fruits with a beginning dark green colouring of blossom-end rot. Secondly, calculations were made of the percentage of the potassium which had been leached out and of the constant of permeability as well. Also in this case there is a higher permeability with low Ca content and a still more marked one at the beginning of the disease.

The formula for the permeation of K does not fit with the experimental data. The cause can be that it is based on a simple diffusion equation, although in our case the diffusion from the outer regions of the tissue is easier than from the inner regions.

The velocity at which the different tissues loose water does not differ much.

The treatment of tomatoes with the permeability-increasing substances ether and decenylsuccinic acid increased the velocity at which the tomatoes began to suffer from blossom-end rot. In other experiments it was demonstrated that these substances really gave a higher permeability for the K-ion.

All results point in the direction that with low Ca content the permeability of the tissues for ions becomes larger and that this can be one of the causes of the disease. This suggestion is supported by the fact that the disease becomes more serious when the membranes are dissolved artificially. The disappearance of the compartments in the cell can be the cause of the lower viability.

Table 3 gives evidence that blossom-end rot appears in the tomatoes with the smallest calcium content, and that this is probably a direct cause of the disease and not a competition for water as some authors assume.

As far as we know has the disease blossom-end rot been explained as a symptom of local Ca deficiency which attacks the membranes of the cell, causes a higher permeability for ions, and may constitute one of the causes of the necrosis of the tissue. Preliminary electron-microscopical research revealed an increasing disorganization of membranes and organelles following a decrease in the Ca level and an appearance of blossom-end rot in the tissue.

Other diseases like bitter pit and tipburn might be ruled by mechanisms similar to that described above.

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