

INVITED REVIEW

A Cellular Hypothesis for the Induction of Blossom-End Rot in Tomato Fruit

LIM C. HO and PHILIP J. WHITE*

Warwick HRI, Wellesbourne, Warwick CV35 9EF, UK

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• **Background** The incidence of blossom-end rot (BER) is generally associated with a calcium (Ca) deficiency in the distal portion of tomato fruits. The visible symptom is a necrotic lesion, which is presumed to be a consequence of cell death and the subsequent leakage of solutes into the extracellular space. Environmental factors that affect either fruit cell expansion or Ca delivery to the distal portion of the fruit influence the occurrence of BER. However, since no absolute, critical fruit Ca concentration for the occurrence of BER has been identified, it is now important to define the role of Ca in fruit cell physiology and to seek the cause of BER at the cellular level.

• **Hypothesis** Here, it is suggested that BER is initiated by a cellular dysfunction in the distal portion of a young fruit during rapid cell expansion. It is proposed that insufficient Ca^{2+} is available for critical apoplastic and cytoplasmic functions when the cellular Ca demand imposed by vacuolation exceeds the Ca delivery to an expanding cell. A local Ca deficiency, therefore, may result in aberrant intracellular Ca^{2+} signals, a weakening of cell walls and a loss of cellular integrity. Ultimately it may lead to cell death and the visible symptoms of BER. Several experimental strategies are suggested to confirm the occurrence of aberrant Ca^{2+} concentrations in cells contributing to BER.

• **Perspective** Many genetic and genomic resources are becoming available for tomato. Ultimately, these will allow genes affecting the occurrence of BER to be identified. Such knowledge will inform breeding strategies to eliminate BER. In the meanwhile, increasing the apoplastic Ca concentration in susceptible fruit tissue should provide a simple and reliable, practical solution for the prevention of BER in tomatoes. It is suggested that current horticultural practices, such as the manipulation of the mineral composition of the feed or the growth environment, are not completely effective in reducing BER because they affect apoplastic Ca concentration in fruit tissue indirectly. Therefore, spraying Ca directly onto young fruits is recommended for the prevention of BER.

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Key words: Annexin, blossom-end rot (BER), calcium (Ca^{2+}), environmental conditions, fruit, mineral nutrition, phloem, tomato (*Lycopersicon esculentum*), xylem.

INTRODUCTION

The symptoms, occurrence and search for the cause of blossom-end rot (BER) in tomato (*Lycopersicon esculentum*) have been described frequently in the scientific literature of the last century (see Brooks, 1914; Spurr, 1959; Saure, 2001). The majority of studies have identified a local Ca deficiency in the distal fruit tissue as the primary cause of BER (Lyon *et al.*, 1942; Ward, 1973; Bradfield and Guttridge, 1984; Adams and Ho, 1992). For this reason, BER was considered to be a symptom of a Ca-related physiological disorder (see Shear, 1975; Bangerth, 1979; Kinet and Peet, 1997). However, the induction of BER in modern glasshouse tomato production is rarely caused by insufficient Ca in the feed. More often, BER occurs in plants with an adequate Ca supply when grown under conditions that either (a) reduce the transport of Ca to rapidly growing distal fruit tissue or (b) increase the demand of the distal fruit tissue for Ca by accelerating fruit expansion (Ho, 1998b). In practice, BER can be prevented by increasing Ca transport toward the fruit by reducing canopy transpiration (Li *et al.*, 2001) or by canopy Ca sprays (Geraldson, 1957; Wilcox *et al.*, 1973; Wada *et al.*, 1996; Ho, 1998a; Schmitz-Eiberger *et al.*, 2002). Nevertheless, since BER may occur in plants and fruits with apparently adequate tissue Ca concentrations, predicting and preventing the occurrence of BER in glasshouse tomatoes

from measurements of their Ca status has not always been effective. This has led to a recent opinion that Ca nutrition is neither a primary, nor an independent factor in the development of BER (Saure, 2001).

The main objections to a primary role for Ca in the induction of BER raised in recent years are (a) that no universal critical Ca level in the BER fruit tissue has been identified (Nonami *et al.*, 1995); (b) that BER can be induced by changing the concentrations of mineral nutrients other than Ca in the feed (Nukaya *et al.*, 1995); and (c) that there is no conclusive evidence for a role of Ca when BER is induced by various environmental stresses (Saure, 2001). However, these objections appear to be based on a paucity of detailed information in the literature. Here are some examples. (1) The inability to find an universal critical Ca level for the induction of BER in fruit could be because, in general (a) Ca has been measured as total Ca (mainly Ca-oxalate and Ca-pectate) rather than as the fraction of Ca relevant to a particular cell function (Minamide and Ho, 1993) and (b) Ca has been measured in whole fruit, or at most in distal tissue, rather than at the cellular level pertinent to cell function. It is possible that a local Ca (or Ca^{2+}) deficiency for individual cells in the distal tissues might be responsible for BER (Schmitz-Eiberger *et al.*, 2002; Suzuki *et al.*, 2003). (2) The induction of BER by changing the concentration of mineral ions other than Ca in the feed can be interpreted as resulting from their effects on Ca^{2+} uptake by roots and transport within the plant, or their effects on

* For correspondence. E-mail philip-j.white@warwick.ac.uk

cellular biochemistry (Geraldson, 1957; Willumsen *et al.*, 1996). (3) The induction of BER by stress factors such as heat, hormones and oxidants does not necessarily exclude the involvement of Ca, since changes in cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_{\text{cyt}}$) are likely to have a role in coordinating the cellular responses to all these stress factors (White and Broadley, 2003). From these counter-arguments, it appears necessary to consider the induction of BER as a cellular phenomenon.

This paper presents a reappraisal of the role of Ca in the induction of BER in tomato fruit. It considers the induction of BER as a cellular event that occurs during rapid cell expansion in a young fruit. How this physiological process might be affected by the responses of the whole plant to the environment and, in particular, how interactions between Ca and other nutritional (i.e. N, K and P), hormonal (i.e. auxin and gibberellins) and stress (i.e. heat, water and oxidative) factors could affect the induction of BER, is discussed.

THE SYMPTOMS AND OCCURRENCE OF BER

Blossom-end rot occurs in tomatoes grown in fields or glass-houses as a horticultural crop. No report on its occurrence in wild species is found. This implies that BER is associated with large fruit growing under conditions favourable for fruit expansion. The induction of BER occurs within 2 weeks of fruit set (Spurr, 1959; Adams and El-Gizawy, 1988), when the Ca concentration in the fruit is at its lowest (Fig. 1). The external symptoms of BER are the collapse of cells in the epidermis and subepidermal parenchyma (Spurr, 1959; Suzuki *et al.*, 2003) and the appearance of a watery, discoloured, necrotic tissue at the blossom-end of the fruit (Fig. 2B and C; Maynard *et al.*, 1957). An internal BER (termed 'black seeds') may also occur when the necrotic region develops in the parenchyma tissue surrounding the young seeds and in the distal placenta (Fig. 2A; Estabrooks and Tiessen, 1972; Adams and Ho, 1992). Internal BER is considered to be either a milder form of the disorder or an earlier phase in the development of external BER. Both are presumed to be consequences of cell death, and the subsequent leakage of cell contents into the extracellular space (van Goor, 1968; Suzuki *et al.*, 2003). The incidence of BER is infrequent, but it can cause a substantial financial loss when it occurs. It can occur in any truss, throughout the seasons (Ho *et al.*, 1993), but is only induced in fruit soon after fruit set, when the relative growth rate of young fruit is at its highest (Ho *et al.*, 1987). This suggests that the induction of BER is associated with rapid cell expansion in the distal fruit tissue. It can be induced by a number of growing conditions, such as low Ca (Raleigh and Chucka, 1944; Maynard *et al.*, 1957; Greenleaf and Adams, 1969; Ward, 1973; Adams and El-Gizawy, 1988; Franco *et al.*, 1994; de Kreij, 1996; Paiva *et al.*, 1998; Adams, 2002) or low P supply (Cerdà *et al.*, 1979; de Kreij, 1996; Ho, 1998a), high Mg (Raleigh and Chucka, 1944; Geraldson, 1957; Hao and Papadopoulos, 2003, 2004), high N (particularly NH_4) (Raleigh and Chucka, 1944; Wilcox *et al.*, 1973; Pill *et al.*, 1978; Pill and Lambeth, 1980; Hartman

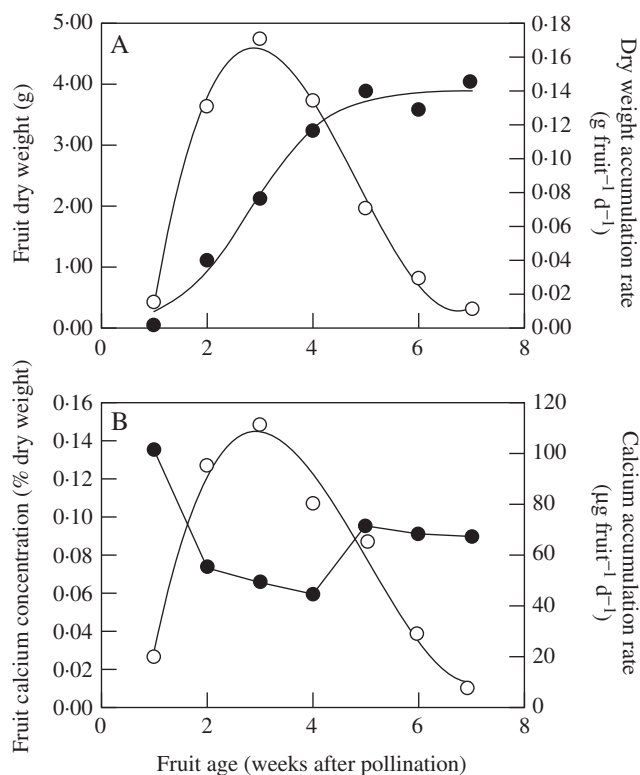


FIG. 1. The time-course of accumulation of dry matter and calcium in a tomato fruit during its development. (A) The accumulation of dry matter (closed circles) follows a sigmoidal relationship with time. Fruit growth rate (open circles) is slow immediately after ovule fertilization during the phase of cell division (Phase 1), accelerates during the phase of cell expansion (Phase 2), and slows again as the fruit approaches its final size (Phase 3). The rate of dry matter accumulation is maximal during the phase of cell expansion. (B) Fruit calcium concentration (closed circles) declines as fruit development proceeds. However, although the rate of Ca accumulation (open circles) is maximal during the phase of cell expansion, the rate of Ca accumulation does not increase as much as the rate of accumulation of dry matter during this phase. Thus, fruit calcium concentration reaches a minimal value during the phase of rapid cell expansion. Data were abstracted from Ehret and Ho (1986a, b) and Ho *et al.* (1987).

et al., 1986; Ikeda and Osawa, 1988; Barker and Ready, 1994; Nukaya *et al.*, 1995), high K (Raleigh and Chucka, 1944; Adams, 2002) or high salinity (Adams, 1991; Adams and Ho, 1992; Willumsen *et al.*, 1996; Cuartero and Fernández-Muñoz, 1999; del Amor *et al.*, 2001; Dorais *et al.*, 2001), drought (Shaykewich *et al.*, 1971; van der Boon, 1973) or water logging in the root zone (Tachibana, 1988), and low humidity (Gerard and Hipp, 1968; Bradfield and Guttridge, 1984; de Kreij, 1996) or high light or temperature (Ho *et al.*, 1993) in the shoot environment. Since these conditions can either inhibit or promote plant growth, BER appears to be unrelated to plant growth rate *per se*. However, BER does appear to be related to fruit growth rate and/or potential fruit size among cultivars (i.e. fruit shape and fruit expansion rate), and there is a clear genetic influence in the susceptibility of different cultivars to BER (Brooks, 1914; Maynard *et al.*, 1957; Greenleaf and Adams, 1969; Adams and Ho, 1992; Ho *et al.*, 1995; Sperry *et al.*, 1996; Cuartero and

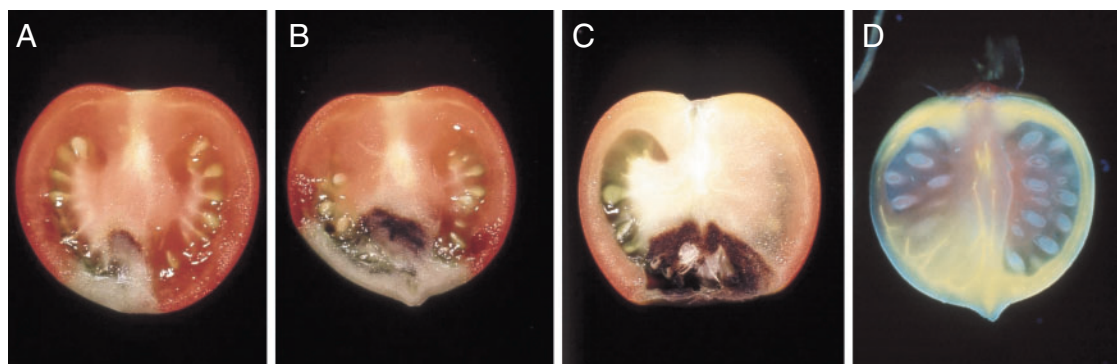


FIG. 2. The symptoms of (A) internal BER, (B) and (C) external BER in ripe tomato fruit, and (D) the development of the xylem network in young tomato fruit. To visualize xylem development, the dye Lucifer Yellow CH was used to stain functioning xylem *in vivo* (Malone and Andrews, 2001). The young tomato fruit appears to possess only two functioning xylem strands in the placental tissue.

Fernández-Muñoz, 1999). Plum tomatoes are more susceptible to BER than round tomatoes, and no BER is ever observed in cherry tomatoes (Ho, 1998a). This has led to the suggestion that the susceptibility of cultivars to BER is related to the differential delivery of phloem-borne leaf assimilate and xylem-borne Ca to the distal end of the fruit in response to the growing environment. Indeed, fruit from cultivars susceptible to BER generally have lower Ca concentrations than those from non-susceptible cultivars, especially immediately post-anthesis (Franco *et al.*, 1994; Willumsen *et al.*, 1996). This may be a consequence of the reduced xylem network in fruit of cultivars susceptible to BER (Belda *et al.*, 1996). A higher incidence of BER is also associated with an earlier onset of ethylene production (Barker and Ready, 1994), cessation of growth and premature ripening.

CALCIUM AND CELL EXPANSION

Since the susceptibility of tomato fruit to BER appears to occur during the early phase of rapid cell expansion, it is pertinent to examine the many critical roles of Ca in this process and to observe that the Ca concentration in fruit decreases substantially during this period (Fig. 1B; Ehret and Ho, 1986a; Cho *et al.*, 1997). It is also significant that Ca is delivered via the xylem to the expanding fruit cells, that the density of xylem vessels decreases during fruit expansion and that there are far fewer, and narrower, xylem vessels at the blossom-end of the fruit than at the proximal end (Belda and Ho, 1993; Ho *et al.*, 1993; Belda *et al.*, 1996). The xylem : phloem ratio also decreases towards the distal end of the fruit. Furthermore, during the critical phase of rapid fruit expansion, although the xylem network in the pericarp tissue increases, there remain only two single functioning strands in the placental tissue (Fig. 2D). These features are thought to be the anatomical reason why BER is initiated in the distal placental tissues.

Hormones such as auxins and gibberellins trigger fruit cells to expand (Gillaspy *et al.*, 1993). Specific changes in $[Ca^{2+}]_{cyt}$ are initiated by these hormonal signals, which effect appropriate developmental responses (White and Broadley, 2003). These signals are generated by Ca^{2+}

influx to the cytosol from extracellular and/or intracellular (endoplasmic reticulum or vacuolar) compartments (White and Broadley, 2003). Thus, Ca has a role in initiating cell expansion, and a local lack of available Ca^{2+} may result in aberrant $[Ca^{2+}]_{cyt}$ signals.

Cell expansion then depends upon the generation of hydrostatic (turgor) pressure, through the accumulation of osmotically active solutes, and the yielding of the cell wall (Nobel, 1999). Although cellulose microfibrils form the basic scaffolding of the cell wall, the rigidity of the cell walls is thought to reside predominantly in the crosslinking of non-esterified pectins by Ca^{2+} (Carpita and McCann, 2000). Cell wall loosening probably proceeds through auxin-induced apoplastic acidification and the activation of endoglycosidases, xyloglucan endotransglycosylases (XETs) and expansins that cleave the load-bearing bonds tethering the wall's cellulose microfibrils to other polysaccharides. As the cell expands, however, pectins in the cell wall become progressively de-esterified through the activity of pectin methylesterases, crosslinked by Ca^{2+} , and branched. This eventually halts cell expansion. In addition, pectins appear to restrict the access of XETs and expansins to their substrates. Thus, Ca has a role in regulating cell expansion, and an insufficient apoplastic Ca^{2+} concentration ($[Ca^{2+}]_{apoplast}$) may result in excessive cell enlargement. It is noteworthy, therefore, that the cells in necrotic regions of BER fruit have ill-formed walls (Suzuki *et al.*, 2003) and that BER appears to be increased in fruit lacking Ca by accelerated canopy transpiration (Gerard and Hipp, 1968; Paiva *et al.*, 1998).

As a cell expands, its plasma membrane and cell wall must increase in area. The incorporation of new material into the plasma membrane and cell wall is also a Ca^{2+} -dependent process and an elevated $[Ca^{2+}]_{cyt}$ has been associated with expansion growth in several cell types (White, 1998; White and Broadley, 2003). It is thought that $[Ca^{2+}]_{cyt}$ controls cell expansion by influencing the incorporation of vesicles containing the materials and enzymes required for membrane and wall construction into the plasma membrane. In elongating root cells, Ca^{2+} influx through hyperpolarization-activated Ca^{2+} channels (HACCs) appears to be responsible for elevating $[Ca^{2+}]_{cyt}$ (White *et al.*, 2002; White and Broadley, 2003). Similar

TABLE 1. The expression of genes encoding annexins in tomato fruit

Annexin	Synonym	Transcript and clone	Expressed in fruit	Reference
AnnLe1	annexin p34	AF079232	Yes	Lim <i>et al.</i> (1998)
AnnLe2	annexin p35	AF079231	Yes	Smallwood <i>et al.</i> (1992)
AnnLe3A		TC128826	Yes	Lim <i>et al.</i> (1998)
		cLEM6A12		TIGR and HRI, unpublished
AnnLe3B		TC122423	—	TIGR
AnnLe4A		TC124457	Yes	TIGR
AnnLe4B		AW650868	—	TIGR and HRI, unpublished
		cLEI14P1		
AnnLe4C		BG642726	—	TIGR
AnnLe5A		TC127030	Yes	TIGR and HRI, unpublished
		cTOA5H24		
AnnLe5B		AI778652	—	TIGR

Each unique tomato transcript has been numbered according to the homologue in *Arabidopsis thaliana* with which it has greatest similarity (White *et al.*, 2002).

When several tomato transcripts were most similar to the same *Arabidopsis* transcript, these were differentiated by an alphabetical postscript.

Sequence data were obtained for tentative consensus (TC) sequences and cDNA clones from the Institute for Genomic Research, USA (<http://www.tigr.org/tdb/lgi/>). Subsequent sequencing of cDNA clones was performed by H. C. Bowen and P. J. White (Warwick HRI).

A putative full-length sequence was obtained for all transcripts highlighted in bold.

channels have been observed in many plant cells, including suspension-cultured tomato cells (Blumwald *et al.*, 1998). Since the activity of HACCs is induced by the presence of reactive oxygen species (ROS) (White and Broadley, 2003), it is interesting to note that ROS are most abundant in fruits during the cell expansion phase (Aktas *et al.*, 2003) and that the production of ROS can be induced by both auxin and gibberellins (Mori and Schroeder, 2004). It is thought that HACCs are formed by annexins (White *et al.*, 2002), and several genes encoding annexins are expressed in tomato fruit (Table 1; Harms, 2003). In tomato, as in other fruit, the expression of genes encoding annexins changes during fruit development (Wilkinson *et al.*, 1995; Proust *et al.*, 1996). These observations indicate the importance of $[Ca^{2+}]_{cyt}$ in coordinating cell expansion.

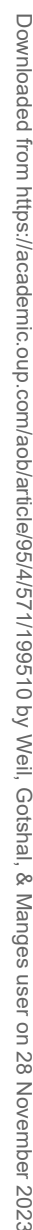
During the initial phase of cell expansion, cells increase the relative volume of their vacuoles (Gillaspay *et al.*, 1993). This results in an increased intracellular Ca demand, because the Ca concentration in the cytoplasm is generally less than that of the vacuole. It has been observed that when Ca movement to the vacuole exceeds the rate at which Ca is supplied to a cell, Ca deficiency symptoms become apparent. So, for example, leaves of plants overexpressing H^+/Ca^{2+} -antiporters that remove Ca^{2+} from the cytoplasm to the vacuole exhibit Ca deficiency symptoms despite having a 'sufficient' Ca concentration (Hirschi, 2001). These symptoms might be a consequence of lowered $[Ca^{2+}]_{cyt}$ or $[Ca^{2+}]_{apoplast}$.

Finally, both $[Ca^{2+}]_{apoplast}$ and $[Ca^{2+}]_{cyt}$ concentrations impact on solute fluxes across the plasma membrane. The integrity of the plasma membrane is maintained by Ca^{2+} binding to negatively charged lipids. In the absence of Ca^{2+} , membranes lose their semi-permeability and become 'leaky' to charged solutes (Kirkby and Pilbeam, 1984). If this occurs, cell death will ensue. The disruption of cell membranes has been observed as an early symptom of BER (Suzuki *et al.*, 2003) and might occur if the

$[Ca^{2+}]_{apoplast}$ around a cell was reduced to a critical level. Hence, it is noteworthy (a) that there is lower Ca^{2+} in the plasma membrane and walls surrounding cells that collapse during rapid cell expansion in BER fruit, and that there is a strong negative correlation between the incidence of BER and the apoplastic Ca concentration in the distal half of tomato fruits (Schmitz-Eiberger *et al.*, 2002; Suzuki *et al.*, 2003), and (b) that BER can be reduced by spraying young fruit with cations, such as Ca or Sr, that stabilize membrane structure (Bangerth, 1973; Ho, 1998a; Schmitz-Eiberger *et al.*, 2002). In addition, the activity of many transporters are modulated either directly by Ca^{2+} or by signalling cascades triggered by changes in $[Ca^{2+}]_{cyt}$. Since controlled accumulation of osmotically active solutes is required for cell expansion, appropriate $[Ca^{2+}]_{apoplast}$ and $[Ca^{2+}]_{cyt}$ must be maintained.

These observations imply that Ca^{2+} is required for several essential functions during cell expansion and it is possible that reduced Ca^{2+} concentrations could be responsible for the initiation of BER in tomato fruit. In summary, since changes in $[Ca^{2+}]_{cyt}$ control cell development, Ca deficiency might cause a cell to develop abnormally. Since Ca^{2+} contributes to cell wall structure by crosslinking pectins, Ca deficiency may lead to cell wall weakness and aberrant cell expansion. Since $[Ca^{2+}]_{cyt}$ coordinates the cellular biochemistry of cell expansion, Ca deficiency might result in morphological defects. Since Ca^{2+} regulates membrane permeability, Ca deficiency may result in abnormal leakage of solutes from cells and cell death.

It is now necessary to test the hypothesis that aberrant Ca^{2+} concentrations initiate BER by confirming that (a) local and/or cellular Ca^{2+} concentrations differ between normal cells and cells that contribute to BER and (b) that perturbation of Ca^{2+} concentration through experimental manipulations affect the incidence of BER appropriately. This could be achieved using transgenic tomato plants expressing the Ca^{2+} -sensor aequorin either in the cytosol



whether cells undergo uncontrolled (necrotic) or controlled (apoptotic) cell death during the induction of BER by comparing the genes expressed in cells contributing to BER with the characteristic biochemical and genetic events that occur during apoptotic cell death (Gechev *et al.*, 2004). To explore the critical role of $[Ca^{2+}]_{\text{cyt}}$ in the initiation of BER, transgenic plants with altered $[Ca^{2+}]_{\text{cyt}}$ homeostasis could also be assayed using this approach. Such plants can be genetically engineered by overexpressing Ca^{2+} -ATPases or Ca^{2+}/H^{+} antiporters under the control of a fruit specific promoter (cf. Hirschi, 2001). Once the involvement of Ca^{2+} in the initiation of BER can be confirmed, the precise mechanism can then be studied.

The induction of BER in young fruit is influenced by a number of environmental factors. These are most likely to exert their effects by affecting the transport of Ca to the fruit and/or the rate of cell expansion (Fig. 3). Although each adverse environmental condition might reduce Ca accumulation and/or accelerate cell expansion in a young fruit through a different physiological process, all are able to reduce the Ca concentration in distal fruit tissue to a critical level. This interpretation is corroborated by the observation that different cultivars show a wide range of responses to growing conditions that induce BER (Adams, 1992; Ho *et al.*, 1995) and the susceptibility of different cultivars to BER appears to be related to the development of the

xylem network and the rate of cell expansion during early fruit development (Ho *et al.*, 1993; Belda *et al.*, 1996). Environmental factors that influence Ca uptake and/or the partitioning of Ca to the fruit include the composition of the feed, its salinity or osmotic strength, and the humidity of the air. Environmental factors that influence the rate of fruit growth, either directly or indirectly by affecting the concentrations of hormones, include water availability in the root zone, light intensity and ambient temperature.

It has been observed that increasing the concentrations in the feed of ions that antagonize Ca uptake promotes BER. In this respect, NH_4^+ (Pill *et al.*, 1978; Barker and Ready, 1994; Nukaya *et al.*, 1995), Na^+ (Adams, 1991; Willumsen *et al.*, 1996; Cuartero and Fernández-Muñoz, 1999; del Amor *et al.*, 2001) and K^+ (Adams, 2002) are particularly noteworthy. Similarly, extreme low or high temperatures (Chong and Ito, 1982; Adams, 1988; Petersen and Willumsen, 1992) or anoxia (Tachibana, 1988) in the root zone reduce Ca uptake and promote BER. In addition, since Ca movement to the xylem is proportional to water uptake (Ho *et al.*, 1995; White, 2001), environmental factors that reduce water uptake, such as extreme root temperatures, anoxia, drought and osmotic stress, also reduce Ca fluxes to the shoot and, thereby, promote BER (Guichard *et al.*, 2001). Indeed, uneven watering or saline conditions in the field or high electrical conductivity (EC) in the feed for glasshouse tomato production have been identified as the most common cause of BER in tomatoes (Cuartero and Fernández-Muñoz, 1999; Franco *et al.*, 1999). Environmental factors that reduce Ca fluxes to the developing fruit, either by diverting the xylem stream preferentially to leaves, such as high canopy transpiration rates (Ho, 1989; Adams and Holder, 1992; Li *et al.*, 2001), or by impairing xylem development within the fruit, such as high EC in the feed (Belda and Ho, 1993; Ho *et al.*, 1993; Belda *et al.*, 1996) also promote BER. It has been suggested that one reason why high N in the feed might promote BER is that it leads to the development of a large canopy and, thereby, augments canopy transpiration (Pill and Lambeth, 1980; Ikeda and Osawa, 1988; Ho *et al.*, 1999).

Cultural practices, e.g. thinning trusses, and environmental factors, e.g. high light intensities and elevated temperatures, which accelerate fruit expansion also promote BER (DeKock *et al.*, 1982; Wui and Takano, 1995). The seasonal trend of BER (Gerard and Hipp, 1968; Ho *et al.*, 1993) and the incidental increase in BER to a sudden change from dull to bright weather (Ho and Grimbly, 1988) are good examples of induction of BER by high light intensities and elevated temperatures. During these periods, fruit expansion is accelerated by high temperatures directly, and high light intensities and elevated temperatures increase canopy photosynthesis and the supply of photoassimilate to the fruit (Ho, 1998b). Under these conditions, it is likely that BER is induced in the rapidly expanding distal fruit tissue because its demand for Ca exceeds the immediate xylem supply (Ho *et al.*, 1993).

Finally, Saure (2001) appears to suggest a direct influence of hormones on the induction of BER. However, although it is possible that various stress conditions produce hormonal imbalances, it is likely that hormones exert their effects

indirectly by influencing cell expansion and plant development. For example, auxins and gibberellins are thought to trigger fruit cells to expand (Gillaspy *et al.*, 1993). The highest concentration of these hormones in fruit occurs prior to cell expansion (El-Beltagy *et al.*, 1976). It is known that the application of auxins and/or gibberellins increases cell division, rapid fruit growth and the incidence of BER (Bangerth, 1973; Castro, 1980). Thus, the acceleration of fruit growth, and the inability of the plant to supply sufficient Ca to rapidly growing fruit, might explain the effects of these hormones on the incidence of BER in the majority of cases. However, blocking polar auxin transport to the fruit can also induce BER. Intriguingly, it has been suggested that this phenomenon might be a consequence of reduced xylogenesis and, therefore, impaired Ca movement to the distal tissue (Battey, 1990). An involvement of ethylene in the induction of BER has also been proposed (Saure, 2001). In addition to its effect on tomato fruit ripening, ethylene has been implicated in initiating responses to wounding and pathogens through $[\text{Ca}^{2+}]_{\text{cyt}}$ signals (White and Broadley, 2003). The precocious production of ethylene is consistent with the necrotic phenotype of BER and is likely, therefore, to be a consequence not a cause of BER. Nevertheless, it is also possible that ethylene, and other 'stress' factors that increase the production of ROS could influence the occurrence of BER through the activation of HACCs and the consequent elevation of $[\text{Ca}^{2+}]_{\text{cyt}}$ and rapid cell expansion.

In conclusion, most environmental factors that affect the incidence of BER perturb the relationship between the rate of cell expansion and Ca accumulation in developing fruit. Further insight on the physiological processes that promote BER could be obtained using the genetic and genomic resources rapidly becoming available for tomato. Firstly, the relative contribution of genetic and environmental factors to the occurrence of BER might be quantified by screening a 'foundation set' of tomato varieties selected to comprise a high proportion of the genetic variation in *Lycopersicon esculentum*. This could then be complemented by the identification of chromosomal loci impacting on the occurrence of BER through quantitative trait (QTL) analysis using genetic mapping populations. Ideally, the parents of these populations would be tomato varieties that show extreme sensitivities to BER, and the populations should show transgressive segregation for this trait. Nevertheless, several mapping populations are already available (e.g. Doganlar *et al.*, 2002; van der Knaap *et al.*, 2002; Causse *et al.*, 2004; Lecomte *et al.*, 2004; Tanksley, 2004), which might be used in the interim. An alternative approach would be to survey the allelic variation in the entire 'foundation set' population and utilize association analysis to correlate phenotypic data with allelic diversity (e.g. Nesbitt and Tanksley, 2002). An appraisal of the genes present in chromosomal loci that influence the occurrence of BER will provide information on the physiological processes contributing to BER and direct further experimentation and breeding programmes. The analysis of chromosomal loci will be facilitated greatly by the sequencing of the tomato genome, which will be completed in the near future (Solanaceae Genomics Network, http://www.sgn.cornell.edu/help/about/tomato_sequencing.html).

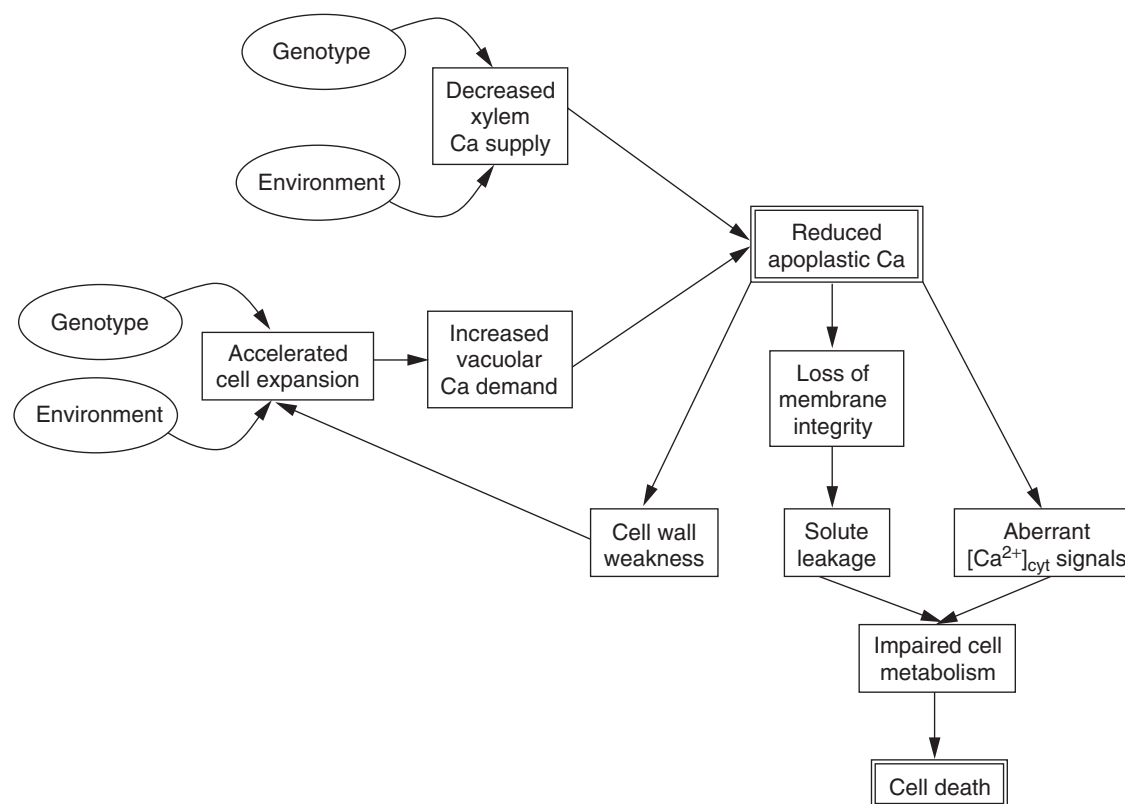


FIG. 4. A hypothesis relating the induction of blossom-end rot (BER) in tomato fruit to effects at the cellular level. Environmental and genetic factors influence the rates of cell expansion and Ca delivery to the rapidly expanding cells in the distal portion of young tomato fruit. An imbalance between Ca supply and cell expansion-led Ca demand leads to a decrease in apoplastic Ca concentration, which impacts on cell wall structure, membrane integrity and cytosolic Ca^{2+} signals. Adverse effects on these critical cellular properties result in uncontrolled solute fluxes and, eventually, cell death.

A WORKING HYPOTHESIS FOR THE INDUCTION OF BER IN TOMATO

All environmental and genetic factors that influence the occurrence of BER in tomato affect either the rate of cell expansion or the delivery of Ca to the young fruit (Fig. 3). It has been observed that BER occurs solely in distal fruit tissue during the initial phase of rapid cell expansion and vacuolation, before any locular tissue is present. Thus, BER occurs during a period of high cellular Ca demand, when fruit growth is accelerated or Ca delivery to the fruit is limited. Recent evidence suggests that BER is initiated as a cellular event, and it has been argued earlier that more detailed information on cellular Ca dynamics during the development of BER could address any substantive argument that Ca is not a primary factor in the development of BER.

This section presents a hypothesis that BER is initiated by a cellular dysfunction in a fruit cell during expansion upon a local, transient Ca-deficiency (Fig. 4). During cell expansion, there is a considerable demand for Ca^{2+} (a) as a structural component of new cell walls and membranes, (b) as a cytosolic signal orchestrating the allometry and biochemistry of cell expansion and (c) as a counter-cation in the enlarging vacuole (White and Broadley, 2003). When Ca is in limited supply, any preferential distribution of Ca to a

particular process might impair cellular function. Thus, excessive Ca accumulation in the vacuole may lead to both aberrant $[\text{Ca}^{2+}]_{\text{cyt}}$ signals and a weakening of cell walls. This is illustrated by the observation that leaves of transgenic plants overexpressing vacuolar $\text{H}^+/\text{Ca}^{2+}$ -antiporters, which remove Ca^{2+} from the cytoplasm to the vacuole, exhibit symptoms of Ca-deficiency (tip-burn) despite having greater tissue Ca concentrations than leaves of wild-type plants (Hirschi, 2001). It was suggested that this phenomenon might be a consequence of altered $[\text{Ca}^{2+}]_{\text{cyt}}$ homeostasis (Hirschi, 2001), but this has yet to be verified. Likewise, it is possible that Ca sequestration in the enlarging vacuoles of a fruit cell expanding during a period of limited Ca supply could starve the cytoplasm or apoplast of the Ca^{2+} required for intracellular signalling or cellular integrity. This might be exacerbated by certain mineral stresses that inhibit Ca uptake or promote the synthesis of organic acids that chelate Ca. A reduction in $[\text{Ca}^{2+}]_{\text{apoplast}}$ could result in impaired cell wall properties, leading to structural weakness and precocious cell expansion, alterations in plasma membrane permeability, leading to solute leakage and unregulated solute fluxes, and aberrant responses to environmental or developmental signals initiated by Ca^{2+} influx. These would ultimately lead to an uncontrolled cell death. Thus, the symptoms of BER,

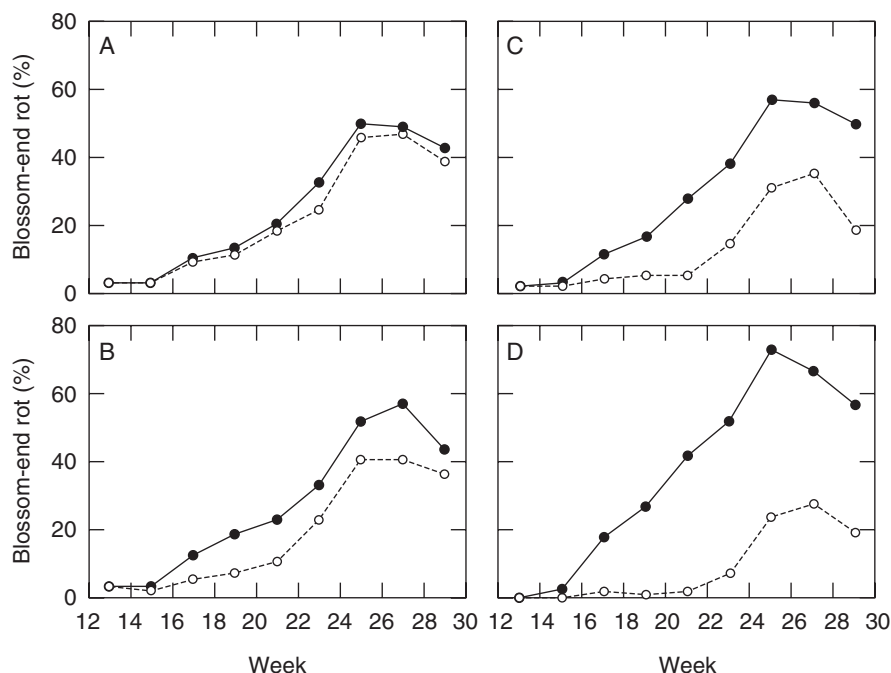


FIG. 5. The effects of nutritional and Ca spray treatments on the incidence of blossom-end rot in plum tomatoes ('Mariella') grown hydroponically. Comparisons of the incidence of BER, expressed as a percentage of the bi-weekly fruit yield, when (A) N in the feed was decreased from 240 (closed circles) to 120 mg L⁻¹ (open circles), (B) P in the feed was decreased from 30 (open circles) to 5 mg L⁻¹ (closed circles), (C) spraying young fruits weekly with 0.5% (w/v) CaCl₂ (open circles; unsprayed = closed circles), and (D) combining all these treatments, with standard N, lowered P and no Ca spray (closed circles) or with reduced N, standard P plus a Ca spray (open circles) (Ho, 1998a).

such as the appearance of a watery, discoloured and necrotic tissue, are consistent with Ca deficiency in the apoplast. Furthermore, it is also consistent with the morphology of individual cells in the BER-affected fruit tissue and the initiation of BER in the unsupported epidermis and subepidermal parenchyma (Spurr, 1959; Suzuki *et al.*, 2003). This hypothesis can explain why the Ca concentration is not necessarily lower in BER-affected tissue than in unaffected fruit tissue, since the vacuolar Ca concentrations in BER-affected tissue could still be high. However, it can be predicted that the Ca²⁺ concentrations in the apoplast and/or cytoplasm of BER-affected tissue will be lower than those of unaffected fruit tissue and this local Ca²⁺ deficiency will initiate BER.

In a horticultural context, BER can be considered simply as a symptom of Ca deficiency in the distal fruit tissue during rapid cell expansion. Thus, BER in a tomato crop can be minimized by spraying Ca onto young tomato fruit (Fig. 5; Wilcox *et al.*, 1973; Ho, 1998a; Schmitz-Eiberger *et al.*, 2002). In the glasshouse, this treatment prevents BER more effectively than other current horticultural practices, such as the manipulation of the mineral composition of the feed (e.g. lower N supply) or the growth environment (e.g. lower canopy transpiration), because it increases the Ca concentration of distal fruit tissues directly. However, this treatment can only be effective when regular Ca sprays are targeted to young fruit before any symptom of BER is observed. The occasional failure of Ca sprays to prevent BER previously may be due to an indiscriminate application to the entire canopy, without any appreciation of fruit

development. An appreciation of how environmental factors affect both fruit growth rates and Ca delivery to the fruit has also informed other management practices to limit BER (Adams, 2002; Ho, 2002). These practices aim to limit growth spurts, and to optimize Ca accumulation, in young tomato fruit. From the forgoing discussion, it is clear that (a) fruit growth spurts can be avoided by preventing momentary high light intensities and temperatures in the canopy, (b) the uptake of Ca²⁺ by roots can be maximized by optimizing the mineral composition of the feed, avoiding high salinity (i.e. <5 dS m⁻¹) or excessive NH₄⁺ (i.e. <10% total N), K⁺ and Mg²⁺ concentrations, whilst maintaining adequate Ca²⁺ concentrations, and/or by preventing extreme root temperatures (i.e. not <14 °C or >30 °C) and drying in the root environment, and (c) Ca delivery to the fruit in the transpiration stream can be maximized by increasing humidity (i.e. by reducing the potential transpiration rate of the crop by one-third), reducing the leaf : fruit ratio, and by avoiding high light intensities and temperatures in the canopy. In addition to informing crop husbandry, an appreciation of fruit Ca physiology at the cellular level can also inform the selection of cultivars for insensitivity to BER. This selection might consider a range of growth characteristics, such as fruit size and shape, fruit : leaf ratio, and the responses of fruit-cell expansion to light and temperature, as well as the efficiency of Ca transport to and within the fruit. It is hoped that, in the future, through the combination of appropriate management practices and the use of BER-resistant cultivars, the occurrence of BER in commercial tomato crops might be eliminated.

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