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ANATOMICAL ASPECTS OF BLOSSOM-END ROT IN THE TOMATO WITH SPECIAL REFERENCE TO CALCIUM NUTRITION

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This report of the general symptoms of blossom-end rot in the tomato describes in detail the anatomical aspects of the disorder. The inception of the rot was observed in field-grown San Marzano tomatoes in relation to the age and development of the fruit. Incipient stages of the rot occurred about 10 to 15 days after anthesis when the fruit was from 38 to 60 per cent full grown in length. During this phase of fruit development the increments of growth in length are very large in relation to the size of the fruit. In general, affected fruits are retarded in length growth.

The externally visible and the deep-seated symptoms of the rot may occur separately or together in the same fruit at the distal end. The symptoms involve a progressive necrosis of the tissue with subsequent partial dehydration of the affected area. Variations in the lesions are described. The highest incidence of the rot involved over 55 per cent of the fruit.

The first indication of the externally visible lesions is the development of brown proteinaceous inclusions in the epidermal and more deep-seated cells of the pericarp. These necrotic cells may collapse and a wound healing response is usually evoked in the adjacent living tissue. Cells showing a wounding response may become necrotic, thus extending the lesion.

Light-yellow to brown proteinaceous inclusions also occur in the cells of the deep-seated lesions, particularly in parenchyma cells associated with the vascular bundles of the placental axis. The cells of affected fruits are often glutted with starch grains. No histological evidence was found to support the view that water stress is a primary cause of the disorder.

Calcium analysis of the fruit confirms earlier reports that the calcium content of the fruit as a whole is low. The distal end of the fruit, the site of blossom-end rot, is particularly low in calcium. Histological and cytological observations of normal and affected fruits are in agreement with some earlier reports on calcium deficiency in the tomato and other plants. This study implicates calcium deficiency in the fruit as the basic cause of blossom-end rot.

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ARTHUR R. SPURR[2]

INTRODUCTION

THE TOMATO (*Lycopersicon esculentum* Mill.) has commanded so much attention in botanical and horticultural literature, it is surprising that no detailed histological account has yet appeared on one of its principal disorders, blossom-end rot, a physiological disorder affecting the fruit. In a general study of the disorder, Brooks (1914)[3] included a few histological observations. Brief comments have also been made by some pathologists (Whitehead, 1896; Smith, 1907; and others) on the tissues of affected fruits.

Blossom-end rot has been reported from virtually all tomato-growing areas in the world. The incidence of the disorder varies considerably with the tomato variety grown (Young, 1942) and with the conditions under which that variety is cultivated. Serious losses are sometimes reported. Geraldson (1956), for example, has observed losses as high as 75 per cent in some fields in Florida. In observations on several varieties of tomatoes grown in the Dutch West Indies, Mullison and Mullison (1948) reported that from 33 to 72 per cent of the fruit was affected. Reynolds (1918) found that in greenhouse-grown tomatoes in North Dakota all the plants in a particular set of observations had some affected fruit and in the majority of the plants all the fruit showed the disorder. Blossom-end rot frequently occurs in California, being especially severe in the San Marzano variety, widely used for processing. The disorder is commonly attributed to various factors, such as water stress, calcium deficiency in the fruit, excess soluble salts in the soil solution, an unbalance of nutrients in the soil, or to combinations of these factors. The purpose of this study is to give a more detailed account of the histopathological aspects of the disorder and some information on the calcium content of the fruit. This may provide a better basis upon which to determine the causal factors involved.

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[3] See "Literature Cited" for citations referred to in the text by author and date.

REVIEW OF LITERATURE

Although a detailed account of the literature on blossom-end rot will not be given here, some background information is essential for interpretation of the histological observations.

The normal anatomy and morphology of the tomato fruit are described by Groth (1910), Brooks (1914), Gardner (1925), Cooper (1927), MacGillivray (1927), Smith (1935), Seaton and Gray (1936), and Murray (1945). These reports as well as the observations made in this study on normal material provided a basis for comparison with fruit affected by blossom-end rot.

The earliest reference to blossom-end rot is the description by Plowright (1881). He referred to the disorder as "black spot" and believed the earlier stages were not due to fungus growth. His figures and brief description clearly indicate blossom-end rot. He found the condition described as "sunscalds" in other horticultural journals. This suggests even earlier accounts of the disorder in the horticultural literature of England and possibly also of the Continent.

A more extensive account was given in 1888 by Galloway, who reported that the disorder was serious and of wide concern at that time. Galloway's work, as well as that of others shortly following, was concerned for the most part with possible mycological or bacteriological causes. However, in 1896 Selby suggested that the rot was associated with insufficient soil moisture. His statement was based on observations during several seasons of different methods of watering tomatoes grown in a greenhouse as well as in the field. Reynolds (1918) hypothesized that an ultramicroscopic organism was involved, but thus far there has been no support for this view. In 1911 Stuckey and Temple clearly established that blossom-end rot is a physiological disorder. Their work also confirmed the belief that a wide range of factors influenced occurrence of the rot. Stuckey and Temple (1911) and others including Brooks (1914), Chamberlain (1933), Stout (1934), and Hoffman (1937, 1953) provide evidence that either irregularity in the water supply or low soil moisture causes blossom-end rot. Robbins (1937) demonstrated that when tomatoes are grown in nutrient solutions of high salt concentration, the incidence of blossom-end rot is markedly increased. This and any other factors seriously restricting the water available to the fruit are interpreted by Robbins as favoring the development of the disorder. Similar results were obtained by Jumelet and Koot (1945).

It is generally recognized, however, that the disorder cannot be attributed to a simple water relationship or to water stress alone. Brooks (1914), for example, reported that continued excessive watering may cause the disorder, and Foster (1939) states that the disorder occurs under conditions of both high and low transpiration. Jumelet and Koot (1945) found that fluctuations of short duration in the water supply have no influence on the occurrence of blossom-end rot. They also reported that a low pH greatly increases the incidence of the disorder.

Wilson and Runnels (1933, 1934) reported that Bordeaux spray increases the incidence of blossom-end rot, possibly by increasing the transpiration

rate. They also found that oil sprays materially decrease the amount of blossom-end rot, possibly by decreasing the transpiration rate.

That vigorously growing plants under a high level of available nitrogen are especially susceptible to blossom-end rot has been shown by many researchers (Chamberlain, 1933; Mack and Stout, 1934; Foster, 1939; Raleigh and Chucka, 1944; Walsh and Clarke, 1945; Leopold and Guernsey, 1953). Brooks (1914) and Spencer and Beckenbach (1949) reported that ammonical fertilizers in particular favor the development of the rot. Stuckey and Temple (1911), however, found that applications of nitrate of soda did not increase the disorder; in fact Brooks (1914) showed that they tended to decrease it.

Recent work has related largely to the calcium nutrition of the plant and to the factors affecting the availability of calcium to the developing fruit. There is some indication in the reports of earlier workers (Brooks, 1914; Bewley, 1923) that calcium may be involved since they suggested that dressings of lime decrease or check the rot. Nightingale *et al.* (1931) found that much of the calcium in the tomato plant is in a "combined" state and that under low-calcium nutrition, the calcium is very slowly re-utilized. They also reported that in low-calcium tomato plants much of the calcium was in the lower half of the stem with only a trace in the upper stem. The opinion is commonly held that under calcium deficiency young growing tissues are unable to obtain adequate calcium from the older tissues of the plant. In solution culture experiments on tomato, Raleigh (1939) found that deficiencies of micro-elements did not induce blossom-end rot. His experiments with solutions lacking the major elements, demonstrated that only in those without calcium did typical blossom-end rot symptoms develop.

Lyon, Beeson, and Barrentine (1942) studied the occurrence of blossom-end rot in sand culture with a wide range of different nutrient solutions varying in the relative proportions of the macro-nutrients. They found that the incidence of the rot was associated only with calcium deficiency. In treatments where the rot was most severe, the fruit was low in calcium content and high in potassium and magnesium. In nutrient culture studies, Walsh and Clarke (1945) found that vigorously growing plants with low calcium in the fruit and foliage showed the most blossom-end rot. On the basis of their experiments at high levels of nutrition they also suggested that high potassium resulted in a depression in calcium uptake with a consequent development of the rot. McIlrath (1950) noted that blossom-end rot developed on plants grown under calcium deficiency in nutrient cultures. He mentions that visible signs of extreme calcium deficiency were evident during the stage of rapid fruit enlargement. The recent work of Ansiaux (1956) on tomatoes grown in sand culture under various double cation deficiencies shows that a deficiency of calcium induces symptoms which he refers to as similar to blossom-end rot. He concludes that an abundance of potassium accentuates the symptoms of calcium deficiency.

Field and greenhouse experiments by Evans and Troxler (1953) using high calcium fertilizer applications, calcium chloride sprays, and calcium gluconate injections into tomato fruit have demonstrated that the incidence of the disorder is either prevented or markedly reduced by these methods.

Geraldson (1955, 1957*a,b*) conducted greenhouse experiments and field tests on the application of calcium in the form of salts to the soil and as foliar sprays on tomatoes. His results indicate that a deficiency of calcium is the fundamental cause of blossom-end rot. He concludes that soluble calcium salts in the soil either may be deficient, or excessive salts may interfere with the calcium nutrition of the plant. Geraldson believes that many of the factors reported to be causal agents of blossom-end rot produce their effect by depressing the calcium available to the fruit.

Mullison and Mullison (1948) found a marked correlation in certain varieties of tomatoes between the application of growth regulators to the flowers and an increase in blossom-end rot. In other varieties an opposite response occurred. They suggest that a hormonal mechanism may be involved. This possibility has some bearing on calcium nutrition in view of the findings of Wuhrmann (1937), Struckmeyer (1949), and Burström (1952) that indicate an important relationship between calcium and growth substances.

Although it is evident that a number of factors modify the incidence of blossom-end rot, two of them merit particular attention in the interpretation of the histological observations. These factors are the effect of unfavorable moisture conditions, particularly water stress, and the effect of calcium deficiency.

MATERIALS AND METHODS

Field Observations and Studies of Fruit Growth. To obtain detailed information on the inception of blossom-end rot relative to the growth of the fruit, 10 plants of San Marzano in a field planting at Davis were selected for observation. Beginning on July 10 and continuing every third day thereafter through September 5, all open flowers were tagged with dated labels in order to obtain a close approximation of the age of the fruit when collected. About 3,200 flowers were tagged on nine plants (one plant was lost by disease). Four collections of fruit were made during the season (July 25, July 31, August 6, and September 14). Each collection except the last was made from three plants from which fruits had not been collected previously. The last collection was taken from all nine plants. At the time of collection all fruits were removed except those having tags for fewer than six days. A total of 3,112 fruit was collected which ranged in age from about six to 54 days from anthesis. The length of each fruit and the presence or absence of external evidence of blossom-end rot were recorded. Each fruit was cut lengthwise to determine whether or not any deep-seated symptoms of blossom-end rot were present. It is difficult to grow the San Marzano variety of tomato in the field without blossom-end rot affecting some of the fruits on each plant. Control material, therefore, for growth studies as well as for anatomical observations was fruit that appeared normal in every respect.

Anatomical Methods. A wide range of ages of normal and affected fruit was collected for study. A special effort was made to find fruits showing incipient stages of blossom-end rot. The material used in this study was fixed in Zirkle-Erliki Fluid (Zirkle, 1934) and was embedded in a water soluble embedding medium (polyethylene glycol). A modified polyvinyl alcohol

aqueous mounting medium described in a previous paper (Spurr, 1957) was used. Most of the slides were stained five minutes in 0.1 per cent brilliant green (Matheson Coleman and Bell, Inc., batch No. 480151) followed by brief staining in 0.1 per cent aqueous thionin (National Aniline Division, Cert. No. NT18). This brilliant green-thionin staining procedure was developed especially for use with the aqueous mounting medium and to differentiate necrotic from normal tissue. In general, necrotic tissue and fatty materials retain the brilliant green after differentiation with thionin. The slides should be observed within a few days after preparation because of a tendency of the thionin to fade. The material shown in figure 5A was prepared by the paraffin method using *n*-butyl alcohol for dehydration.

Microchemical Tests and Calcium Analysis of the Fruit. Unstained sections of tissue prepared by the polyethylene glycol embedding method were used for the microchemical tests. The sections were held on slides with Haupt's adhesive. The material is apparently suitable in tests for any substances not removed by the fixing fluid and washing procedure or by the polyethylene glycol. The latter is incompatible with a number of substances in plant tissue. Lipoids in particular remain apparently unchanged by contact with polyethylene glycol. The use of polyethylene glycol in the processing procedure also has the advantage that the cells show few artifacts and favorable material can be cut as thin as 3 μ in thickness without difficulty. These qualities permit better interpretation of the cytological details than when much thicker free-hand or freezing microtome sections are prepared from fresh material. The specific microchemical tests used are given in the results.

A separate field planting at Davis of San Marzano tomatoes was used as a source of material to determine the calcium content of different portions of the fruit. Four classes of actively developing fruits, about one-third grown in length, were established as follows: 1) normal fruit; 2) fruit with incipient blossom-end rot; 3) fruit with more advanced blossom-end rot; and 4) normal fruit injected with calcium gluconate (one hypodermic injection each at mid-level of 0.10–0.25 ml of 2 per cent of calcium gluconate). The fruits were harvested when mature. In this exploratory experiment only three to eight fruits were used to make a composite sample of each class. Samples for analysis were prepared by cutting the fruits into thirds: the basal or stem end, the middle portion, and the distal or blossom-end. The calcium content on a dry-weight basis was determined by flame spectrophotometry (Beckman quartz spectrophotometer, model DU with a special photomultiplier unit for flame analysis; see Brown *et al.*, 1952).

RESULTS

General Organization of Fruit and General Location and Appearance of Lesions. The fruit consists of a bicarpellate superior ovary with an elongated axial placenta to which numerous seeds are attached (fig. 2A–D). The septa of the carpels divide the ovary into two locules. The highly parenchymatous portion of the placenta surrounding the seeds largely fills the locules, although air spaces may remain near the base and apex of the locules at maturity.

Our observations are in general accord with those of Diotallevi *et al.* (1947), who gave a brief account of the symptoms of blossom-end rot in the San Marzano variety. Figure 1A shows the general appearance of the disorder in a single cluster of fruit. Some of the variations occurring in the externally visible lesions are shown in figure 1B. The lesions differ widely in size, but their form is generally circular. The mature lesions may also be

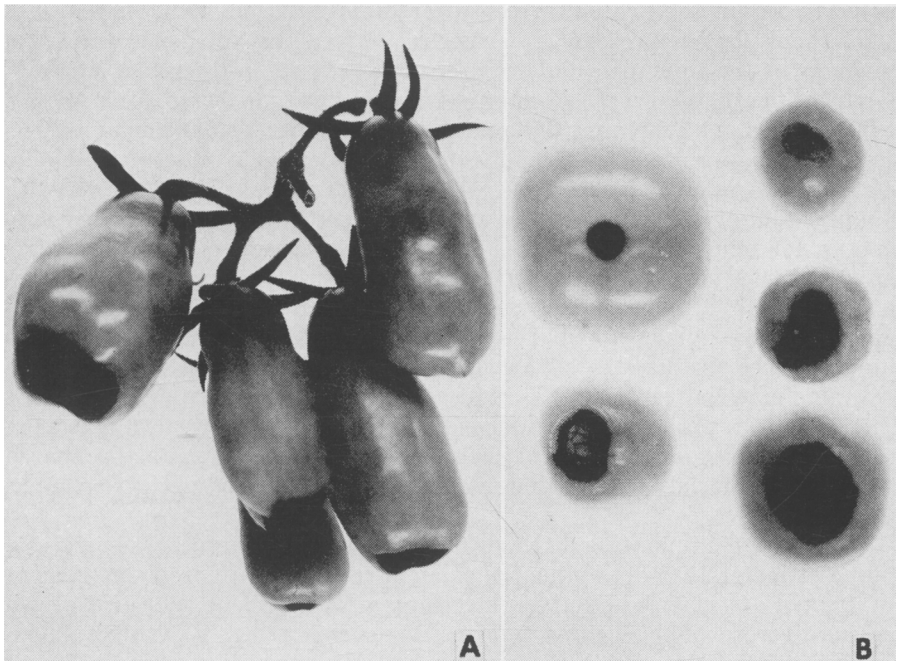


Fig. 1, A, B. Immature San Marzano fruits affected by blossom-end rot. *A*, Cluster of five fruits showing four with advanced symptoms; one normal fruit. *B*, Distal-end views of fruits showing variations in the superficial appearance of blossom-end rot lesions. *Upper right*, a moderately early lesion with slight discoloration of tissue. *Middle right*, an advanced darkened lesion asymmetrical in form. *Lower right*, a large advanced lesion. *Upper left*, a relatively small but advanced lesion on a full-grown fruit. *Lower left*, an advanced lesion, asymmetrical in position, not involving base of the style. The underlying network of necrotic vascular tissue is evident. (Photographed with an Eastman Kodak portra + 3 lens over the camera lens; a Wratten filter, No. 58, green, was used for A; all X1.0).

quite asymmetrical. They are usually centrally located with reference to the axis of the fruit, but in some cases the lesion is acentric in position and does not involve the region at the base of the style.

When fruits are cut lengthwise (fig. 2A-D) it is evident that two general areas in the fruit may be initially and independently affected: 1) the distal end of the placenta, and 2) the pericarp at the distal end of the ovary. In the first case (fig. 2A) the fruit may show no external evidence of the disorder, and the apex of the fruit is normal in form. In the second case (fig. 2B) the lesion is the familiar externally visible symptom of blossom-end rot. The apex of the fruit is often flattened or slightly depressed. The two areas

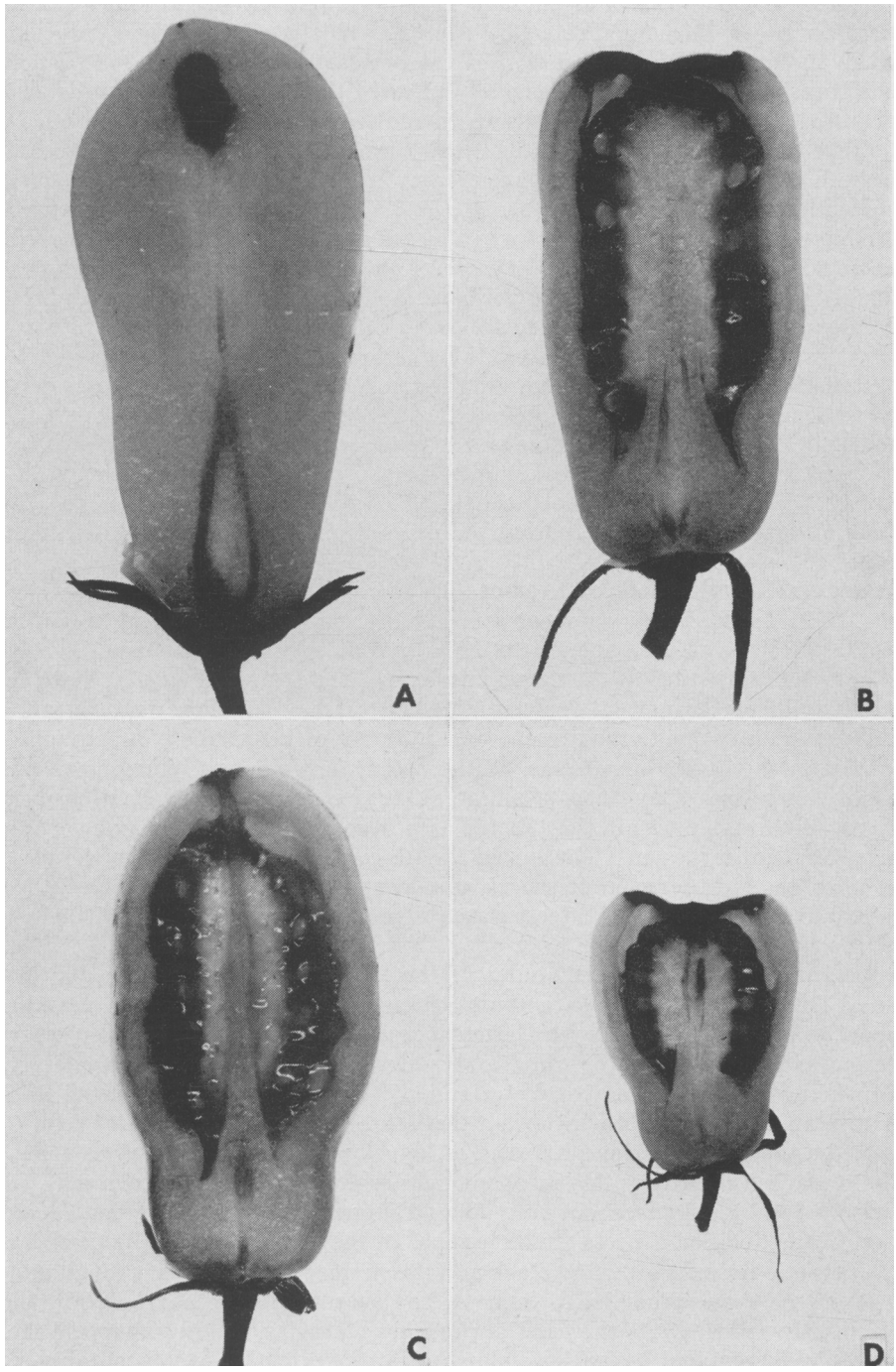
may both develop lesions in the same fruit (fig. 2C) leaving the intervening tissue between them unaffected by necrosis. This condition seems to occur when the externally visible lesion of the pericarp is small (fig. 2C). When both areas are moderately to severely affected the intervening tissue is also necrotic. In spite of these variations in the area affected, one characteristic feature of the disorder is that the distal tissues of the pericarp or placenta or both are the primary sites for necrosis.

Incidence of Blossom-end Rot. Of the 3,112 fruits tagged and measured during the season, a total of 304 was affected by blossom-end rot, which gives an incidence of 9.76 per cent. In some of the collections, however, the incidence of rot was as high as 55.6 per cent. Although the collections with high incidence of the rot appeared to follow periods of high field temperatures, this study was not designed to test this or the relationship of the disorder to cultural practices. Since fruits were collected at different stages of development some were very likely collected before symptoms were evident and, thus, the incidence of blossom-end rot appears lowered somewhat. Among the affected fruit, 25.0 per cent showed lesions, not externally visible, affecting the distal portion of the placenta; 22.7 per cent showed superficially visible lesions affecting the pericarp only; and 52.3 per cent exhibited both types of lesions.

General Development of Lesions. The first external lesions visible under the dissecting microscope appear somewhat irregular in shape and are quite small, affecting an area about 1 to 2 mm in diameter. They are usually very slightly depressed below the normal surface of the fruit and have at first the green color of the normal fruit surface. Later they are more transparent; this is evidently due to the presence of fluid within the intercellular air space system in the underlying tissue. With further development, portions of the fluid-soaked area may show a slight darkening, or separate small brown flecks may develop within the affected area. Each affected fruit may have one or several initial lesions. The initial lesions usually do not involve the base of the style, but occur one to several mm away from it.

In advanced stages the affected area is more extensive, incorporating all of the initial sites, if more than one. At this stage the lesion has a wilted appearance whereas the remainder of the fruit is firm. The lesion may be quite irregular in shape. In a fruit 3.0 cm long the affected area may be about 0.7 cm by 0.5 cm. As the lesion increases in size its surface generally becomes light brown and wrinkled and moderately soft in texture. In actively advancing lesions the entire distal end of the fruit becomes affected and within about a week after inception the lesion attains its full size.

After the lesion becomes arrested in development its periphery is sometimes marked by a very fine inconspicuous white line which in turn may be surrounded by a transparent ring about 1.0 mm wide. There is much variation in the detailed aspects of the lesions. In the final stages of the disorder the necrotic tissue is brown to black and the surface of the lesion is tough and leathery or even rather hard. Some of the necrotic tissue may be white or essentially colorless. As the fruit approaches maturity, the periphery of the lesion is surrounded by an irregular and often extensive yellowish area, a condition which is indicative of premature ripening of this portion of the


A
B
C
D

fruit. This yellowish area then turns red, whereas the more basal portions of the fruit remain green. In mature fruit the lesion is generally somewhat dried so that it is often sunken below the nonnecrotic tissue around it.

The earliest deep-seated symptom noticed before obvious browning occurs is a pithiness, the tissue appearing drier and whiter than normal. The development of the tissue is arrested and in slightly later stages small scattered groups of brown necrotic cells appear. The most predominant deep-seated lesions affect the parenchyma tissue surrounding the young seeds. The seeds are retarded in development and in later stages they may become necrotic. Figure 2C shows two seeds in the upper right-hand locule that have been arrested in their development by the proximity of a lesion. The seeds at lower levels in this fruit have attained their normal mature size.

Primary lesions affecting the axial tissue of the placenta are less common than those affecting mainly the parenchyma tissue around the young seeds. They are generally at the distal end, but they may be quite deep-seated. In one case, for example, the distal end of an internal lesion 0.4 cm long was 1.3 cm from the base of the style in a fruit 3.0 cm long. Such deep-seated necrosis is more characteristic of advanced stages. When the lesions of the placental axis advance downward the tissue primarily affected is associated with the vascular bundles (fig. 2D). This involvement of the vascular tissue is a secondary development. They appear unaffected in the initial stages of the disorder when the epidermis or parenchyma tissues at the distal end of the fruit become necrotic. In severe cases a relatively large mass of necrotic tissue occurs in the fruit.

Relation of Blossom-end Rot to Fruit Growth. The incipient stages of the disorder were found to occur in fruit ranging in age from about 12 to 15 days after anthesis. In fruits older than 15 days only the more advanced stages are present. Since the earliest macroscopically observable symptoms were seen in fruits at least 12 days old, one can assume that the disorder is initiated in fruits younger than this. However, fruit nine days old showed no symptoms of the disorder; thus it is estimated that the earliest phase of the disorder in this study occurred in fruit 10 to 15 days old (fig. 3). At this age the mean length of the fruit is 2.7 cm to 4.2 cm or about 38 to 60 per cent full grown in length. Although early symptoms occur only in this age range the

Fig. 2A–D. Median longitudinal views of San Marzano fruits showing their general organization and the areas primarily affected by blossom-end rot. The fruits generally have two carpels with an axial placenta. *A*, Mature fruit (red in color) cut in the plane of the septa separating the two locules. This fruit is normal in form and shows no external evidence of blossom-end rot, but the axial portion of the placenta at the distal end has a deep-seated lesion. *B*, Fully grown green fruit cut in the median plane of the two carpels. Note the depressing effect of the large, externally visible lesion on the form of the fruit. Upper portion of the placenta is apparently secondarily affected. *C*, Fully grown green fruit cut as in *B* showing two primary lesion sites, an externally visible lesion at the apex of the fruit and a deep-seated lesion involving primarily the thin-walled parenchyma surrounding the young seeds at the distal end of the placenta. *D*, A green fruit cut as in *B* that has apparently stopped growth in length, showing the marked effect of blossom-end rot on the development of some fruit. Dark vertical streaks in the central portion of the placenta are brown necrotic tissue associated with the vascular bundles. (Photographed with an Eastman Kodak portra + 3 lens over the camera lens; a Wratten filter No. 29, red, was used for A and No. 58, green, was used for B–D; A–C X1.3, D X1.0.)

size of some of the individual fruits in which new symptoms occur is somewhat less or greater than the mean lengths indicated in figure 3. The increments for increase in length of the normal fruit are of about the same magnitude for each interval from 6 to 25 days after anthesis. The length of the affected fruit, however, is slightly less than that of the normal at 18 days. The depressed rate of growth in length of the affected fruit appears to coincide with the onset of the disorder. The affected fruits continue growth,

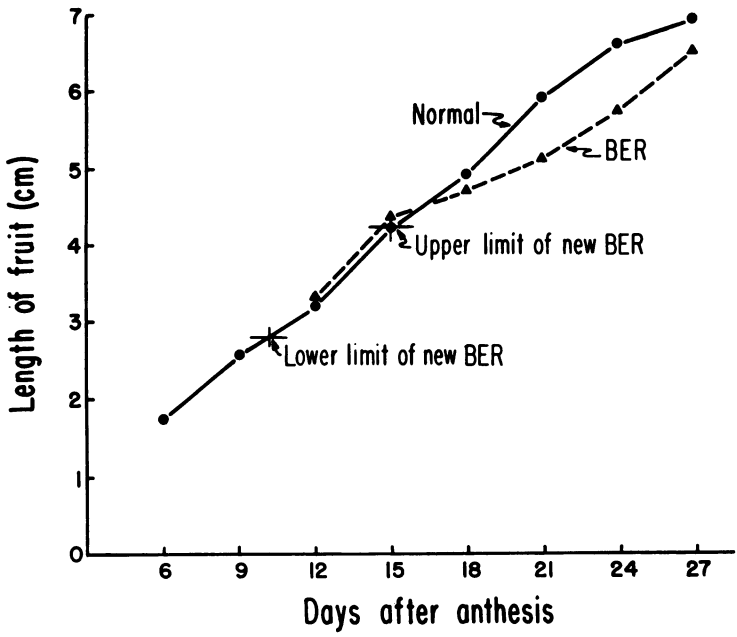


Fig. 3. Growth curves showing increase in mean length of normal and blossom-end rot (BER) of San Marzano tomato fruits. Lower and upper limits for new BER are estimated on the basis of age of the fruit. Actual length of individual fruit with new BER may vary from these limits since the curves are based on mean length determinations.

but their eventual length is generally less than normal (fig. 3). In some cases (fig. 2D) the fully grown fruit is much smaller than normal. The curve for growth in length of normal fruit is basically similar to the curves reported by Gustafson (1926, 1927*a*) in his detailed studies of volume and green weight changes in tomato fruits.

Figure 4 illustrates the amount of growth during each three-day period relative to the size of the fruit. Growth is much greater during the earlier stages of development than during the later stages. During the 9- to 15-day periods, for example, the increments are on the order of 25 to 35 per cent for each three-day interval, but during later stages the percentage of increase in length markedly decreases as the fruit matures. This feature of growth is not shown by the more conventional growth curve. Since the inception of blossom-end rot occurs only during a relatively active phase of

growth, it is evident that the disorder is in some way correlated with fruit development.

Needham (1931) examined the various methods of plotting the development of organisms against time. He emphasized that the percentage of growth increase during an interval should not be related to the size of the organism at the beginning of the interval. Growth is a continuous process and its rate at any instant is functionally related to the status of the indi-

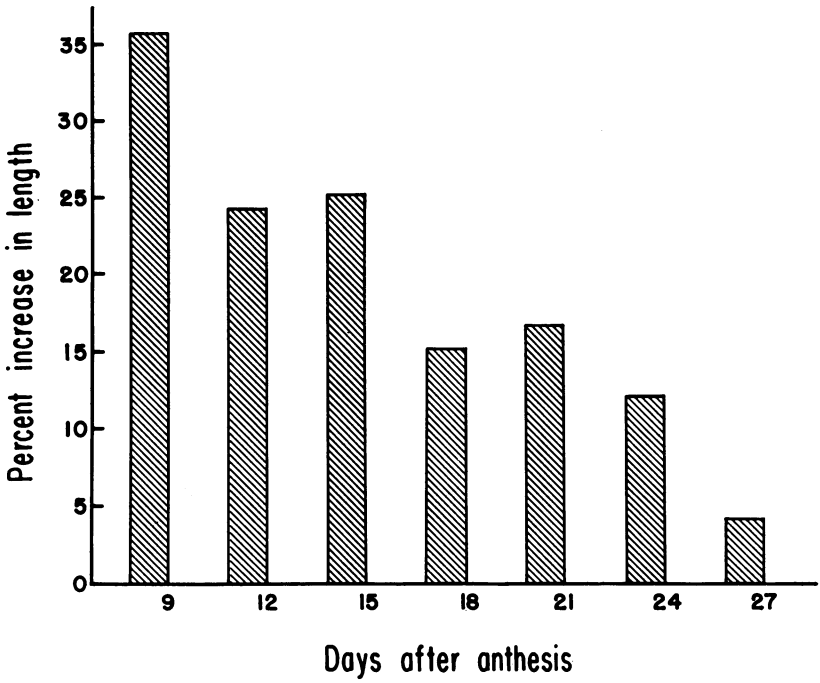


Fig. 4. Graph showing increments, expressed as per cent, for increase in mean length for three-day periods ending on the days indicated along the horizontal axis for normal San Marzano tomato fruit. Note that the increments during the earlier stages of growth are relatively large with respect to the length of the fruit. See text for further explanation.

vidual at that instant and not to some point in the past. In the preparation of figure 4 the following procedure was used. The mean length of fruit of each age was determined and the successive increments calculated. An intermediate value at two thirds of the difference between the beginning length and final length was determined for each interval. The two-thirds value was then expressed as the percentage of increase over the length of the fruit at the beginning of the intervals. This method avoids the extreme values that are obtained when either the beginning or end point of an interval is used as the basic reference point to express the percentage of growth increase.

Histopathology of Incipient Stages. The earliest changes in the tissues affected by blossom-end rot were evident before any collapse or breakdown of the cells was observed. Plate 1A shows that the epidermis and under-

lying parenchyma cells of the pericarp have maintained their form, yet, brown inclusions are present in the protoplasts of some epidermal cells. The color of the inclusions is not due to the staining procedure used. The granules appear to be associated with the cytoplasmic strands and do not appear to bear any particular relationship to the nuclei. The cells underlying the epidermis show no obvious evidence of abnormality. Plate 1B illustrates a slightly later stage. Brown inclusions are evident in a number of the epidermal cells. The cytoplasm appears in an advanced state of disorganization. No cytoplasmic strands are evident; the nuclei are deeply stained and seemingly in abnormal positions, that is, in some cases appressed against the inner wall of the cell. A few cells in the third and fourth layers of the pericarp are partially collapsed and the nuclei in two of them are deeply stained and apparently abnormal in shape. The collapse of the internal cells of the pericarp results in a slight depression of the epidermis. This is one basis for the first visible indication of blossom-end rot lesions on the fruit. As the disorder progresses more cells of the pericarp become involved. Groups of cells collapse or partially collapse and the epidermis becomes distinctly depressed (plate 1C). All of the cells in this figure are lacking in normal cytoplasmic organization. Cytoplasmic strands are absent in contrast to the earlier stage shown in plate 1A. Masses of deep-staining material are particularly evident in some of the epidermal cells. Such masses appear to be involved with the degradation of the nuclei. The cytoplasm of partially collapsed cells appears to be coagulated, and light yellow granules are evident in some of the cells. Gum-like deposits occur in the affected tissue, for example, the blue-green staining material in the sixth layer of plate 1C.

The necrosis is not necessarily limited to the epidermis and the immediately underlying layers of the pericarp. Plate 1D shows the collapse of a group of cells at about the mid-point in the wall of the fruit. The large cells at the left of the collapsed cells show no evidence of collapse, but their cytoplasm appears disorganized. At the extreme upper left are cytoplasmic strands associated with a nucleus. The light yellow granular material in this cell suggests, however, that it may be in the initial stages of necrosis. Plate 2A shows an advanced stage in the necrosis of the inner layer of the pericarp. Some of the adjacent cells of the pericarp show earlier stages of necrosis—a more deeply staining quality of the nuclei, and disorganization of the cytoplasm. The thin-walled parenchyma tissue at the left is that part of the placenta surrounding the seeds.

Collapse of the necrotic tissue is not necessarily a constant feature of the disorder. The cells with green-staining contents in plate 2B are evidence of an extensive necrosis of the pericarp in a moderately young lesion, yet there is no indication of collapse of the affected cells.

Histopathology of Advanced Stages. As the lesions increase in size, the cells at the periphery of the necrotic tissue frequently show a wounding response. This is illustrated in plate 2C which shows files of cells which have been derived by a series of divisions periclinal with respect to the mass of collapsed necrotic tissue. Mitotic figures were not observed. This response in part accounts for the banding effect that is often present and visible externally at the periphery of blossom-end rot lesions. The wounding response

PLATES

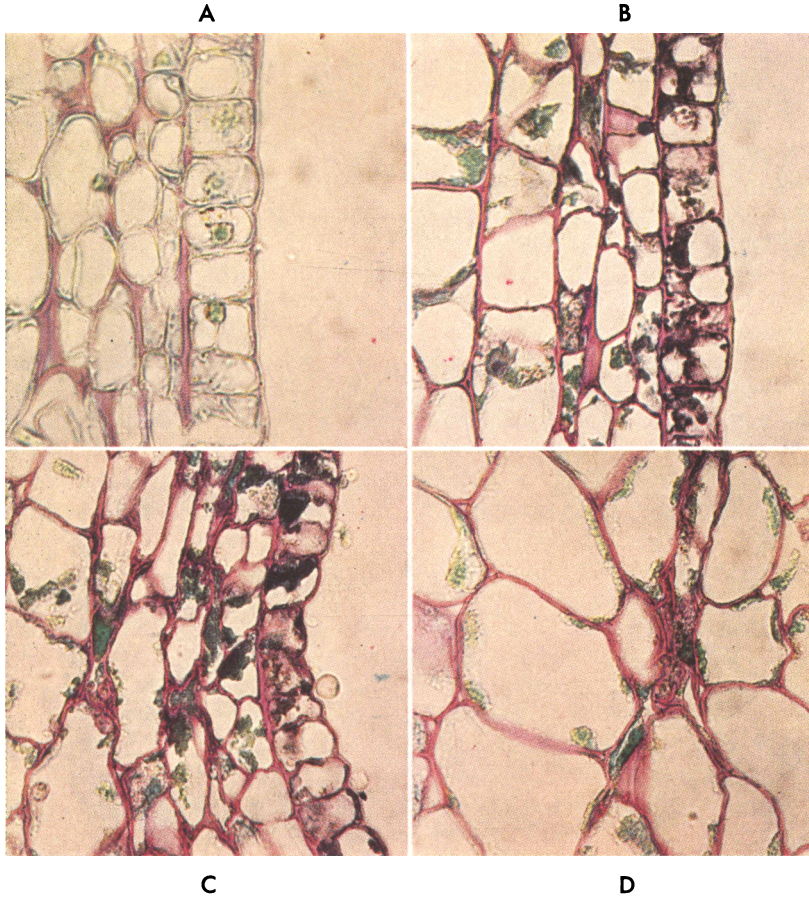


Plate 1 A–D. Longitudinal sections of the pericarp of the distal end of young fruits showing early stages of blossom-end rot. *A*, Section of a fruit 2.3 cm long showing the brown proteinaceous inclusions in the protoplasts of some epidermal cells. This is the first cytological evidence of blossom-end rot affecting the pericarp. The cells were probably living at the time of fixation. Note the natural appearance of the protoplasmic strands in the cells due to the use of the polyethylene glycol embedding technique. *B*, Section of a fruit 2.5 cm long showing disorganization of the protoplasts of the epidermal cells, brown inclusions in the epidermis, and the collapse of some underlying cells of the pericarp. *C*, Section from a fruit 2.6 cm long showing a more advanced stage. The collapse of the underlying tissue has caused the depression of the surface of the fruit. *D*, Section from the same fruit shown in *C*. Cells at a mid-level in the pericarp have become necrotic and have collapsed (All X410). (The color photomicrographs in this report were made with Kodak Ektachrome daylight 120 film, Wratten light filter No. 80 over the source, and process kit E-2).

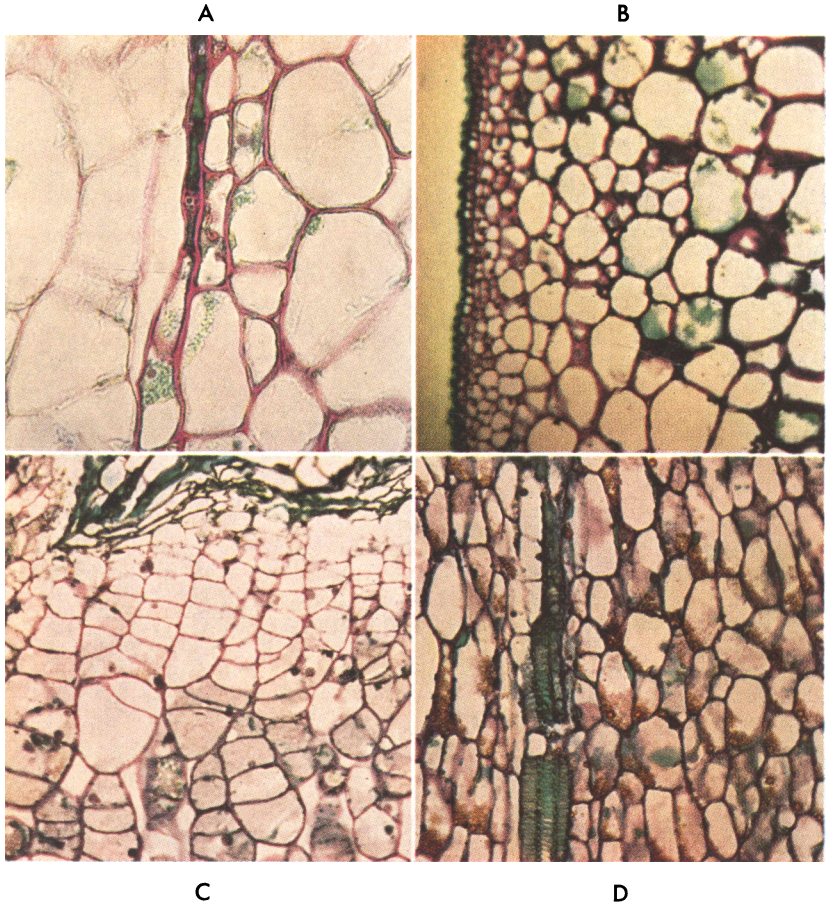
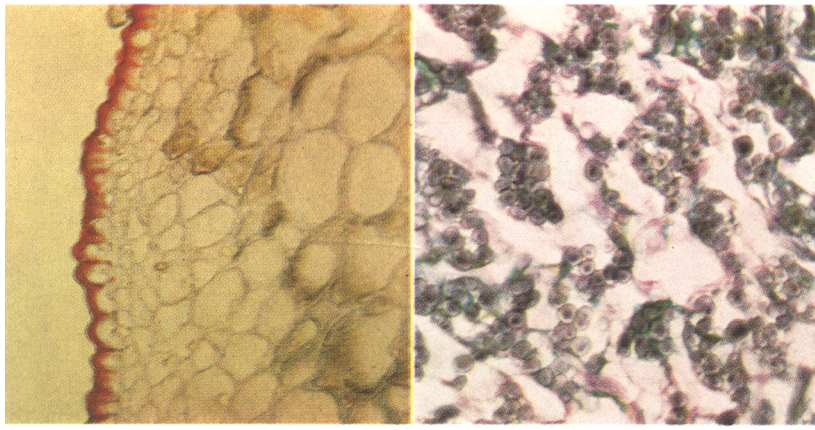


Plate 2 A–D. Longitudinal sections of tomato fruits with blossom-end rot. *A*, Section of a young fruit 2.4 cm long showing the inner portion of the pericarp on the right and parenchyma cells of the placenta on the left. Note the necrosis and collapse of the cells of the inner epidermis of the pericarp. *B*, Section of the pericarp of a fruit 2.5 cm long showing necrotic cells with green staining contents and the absence of any collapsed cells. Note that the cells near the lesion do not show a wound healing response by division. *C*, Pericarp of a fruit 3.2 cm long showing the lesion above and an extensive region of wound healing below. All of the latter region is also necrotic. One indication of this is the seemingly amorphous contents filling many of the cells in the lower portion of the figure. Note the common derivation of groups of cells in the wounding response. *D*, Necrotic tissue of the placental axis of a fruit 2.6 cm long showing the numerous brown proteinaceous inclusions in parenchyma cells associated with the vascular tissue. Note that the inclusions have accumulated in the same relative positions in each cell (A X410; B, C X55.5; D X122).



A B

Plate 3 A, B. Longitudinal sections of fruit affected by blossom-end rot. *A*, Pericarp of fruit 2.5 cm long showing necrotic cells with fine-grained contents. The section is stained with oil red 0 in propylene glycol. Note the absence of any accumulations of lipoidal materials in the necrotic cells and the deeply staining cuticle and epidermal walls. *B*, Necrotic cells of the pericarp of fruit 3.2 cm long showing that some are glutted with starch, a condition possibly due to calcium deficiency. The protoplasmic contents of the starch-filled cells have a strong affinity for the blue-green stain, an indication of necrosis. The starch grains have been lost from some cells during processing (A X80; B X122).

at the periphery of the lesion may be very extensive. The tissue undergoes hyperplasia particularly at the edge of lesions inside of the locule, forming masses of callus tissue. Affected tissue surrounding the seeds that have been arrested in development may also develop extensive callus.

In some cases the wound meristem that is formed has the characteristics of a typical phellogen. In figure 5A the necrotic tissue is at the top of the figure. Below it is a phellogen layer in which the cells have undergone about two divisions each, periclinal with reference to the mass of necrotic tissue. This type of wounding response is quite weak and appears to occur late in relation to the development of some lesions.

Even though a wound periderm is extensively developed, necrosis and collapse of cells may occur in the tissue involved in the wounding response. In plate 2C all of the tissue is in fact necrotic. One indication of this is the seemingly amorphous contents filling many of the cells in the lower part of the figure. The necrosis of the tissue in which numerous divisions have occurred advances the lesion toward the base of the fruit. The tissue at the periphery of the lesion may in turn show a wounding response. Although recently divided cells in tissue adjacent to the lesion are very common, some lesions evoke no response of this type (plate 2B).

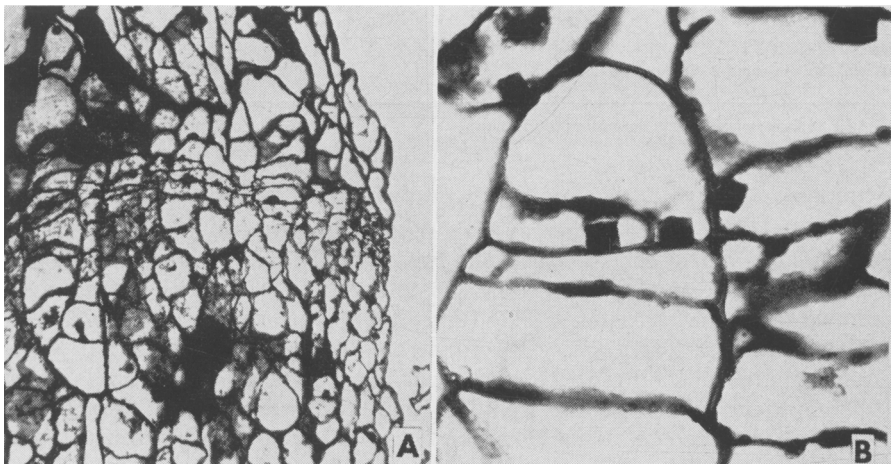


Fig. 5 A, B. Longitudinal sections of tomato fruit with blossom-end rot. *A*, Pericarp of mature fruit 6.3 cm long showing that the wounding response is restricted to a layer of cells in the tissue just below a lesion. This is the only figure showing material processed by the paraffin method. *B*, Cuboidal protein crystalloids in the wound meristem cells near a lesion of a fruit 3.5 cm long. (A X128; B X410).

When necrosis occurs in the placenta either the parenchyma surrounding the seeds or the more axial tissues may be affected. Necrotic parenchyma cells from the axis of the placenta are shown in plate 2D. They are associated with the vascular bundles and contain characteristic masses of small light-yellow or brown inclusions as well as a few larger brown spherical inclusions. The inclusions are clumped generally at one end or side of the cell and the relative position of the clumps in nearby cells is similar. However, the rela-

tive position of the clumps of granules in other portions of the same lesion is often very different. In addition to the brown inclusions the cells contain a finely granular, light-purple staining flocculent material, small protein crystalloids, green staining gum-like masses, and remnants of nuclei and plastids. Details in the cytological origin of these various features were not followed. The affected cells of the placental axis generally do not collapse. It is of interest that the adjacent living cells do not show a wound periderm response, a feature so common to the pericarp.

Necrotic cells of the thin-walled parenchyma around the seeds frequently collapse and the development of the tissue as a whole including the associated seeds is depressed. The effect on the seeds is secondary and the disorder is not due apparently to a failure in pollination or fertilization.

TABLE 1
CALCIUM CONTENT OF MATURE SAN MARZANO TOMATO FRUIT*

Classes of fruit at start of experiment†	Calcium in grams per 100 grams dry weight of different portions of the fruit		
	Basal	Middle	Distal
Normal	0.06	0.03	0.02
Incipient BER	0.10	0.02	0.03
Advanced BER	0.09	0.03	0.02
Normal fruit injected with Ca gluconate	0.10	0.04	0.03

* Using flame photometric determination method.
† See section on materials and methods for details of classes.

When the seeds become necrotic, one of the first sites in which breakdown occurs is the outermost layer of cells of the integument, especially in the proximal regions of the seed near the funiculus. In more advanced stages of the disorder the two or three outermost layers of cells of the integument collapse. Eventually the entire seed may become necrotic.

Microchemical Tests. Plate 3A illustrates the results obtained in staining for lipoids with oil red O in propylene glycol. The outer and radial walls of the epidermis show strong lipoid accumulations by their affinity for the red stain. The necrotic tissue, however, shows only slight indications of fatty materials. The phloroglucinol and HCl test for wound gums (Hewitt, 1938) was used on some sections. In some necrotic cells the amorphous material gave a pink to red positive test and it also gave a positive light-yellow xanthoproteic reaction with concentrated HNO_3.

Starch grains are present in both normal and affected fruit. In the latter, however, many of the necrotic cells are gorged with them (plate 3B). The grains appear normal in size, show no evidence of erosion, and give the characteristic blue color with I_2KI.

Protein crystalloids are a common feature of the tissue adjacent to blossom-end rot lesions (fig. 5B). They appear to be especially abundant in cells that have divided as a response to wounding. The crystalloids appear to be cuboidal in form. They are insoluble in glacial acetic acid, concentrated H_2SO_4, and 25 per cent HCl. A deep-brown stain is produced with I_2KI and

a pink color with 2 per cent acid fuchsin. They also give a positive test with Millon's reagent and a positive yellow xanthoproteic reaction with concentrated HNO_3.

The brown inclusions in the parenchyma tissue associated with the vascular bundles of the placenta are insoluble in glacial acetic acid, concentrated H_2SO_4, and 25 per cent HCl. They show no staining with I_2KI, 2 per cent acid fuchsin, and oil red O. No color reaction was obtained with $FeCl_3$, although the brown granules resemble inclusions in other plants that are usually denoted as tannins. They are negative with Millon's reagent, but give a yellow xanthoproteic reaction with concentrated HNO_3. This reaction, particularly when a base is added, causes them to be more deeply colored.

Calcium Analysis. Table 1 summarizes the results of one experiment on the general distribution of calcium in mature fruit. Different portions of the fruit show a pronounced difference in calcium content on a dry-weight basis. The significant point in the data presented here is that the basal portion of the fruit contains much more calcium than the middle and distal portions (0.06 to 0.10 gm/100 gms *vs.* 0.02 to 0.03 gm/100 gms, respectively). In this limited test there appeared to be no difference in calcium content between normal fruit and fruit affected with blossom-end rot. The injection of small amounts of calcium gluconate into developing fruits did not affect their calcium content at maturity. None of the injected fruits or fruits selected as normal at the beginning of the experiment developed blossom-end rot during the course of the experiment.

DISCUSSION

Although it is evident from the literature that a number of factors influence the occurrence of blossom-end rot, the foregoing results will be considered here only in relation to two principal causes suggested for the disorder, water stress and calcium deficiency.

Water Stress. Very little histological evidence was found showing water stress in the fruit to be a primary cause of the disorder. The early internal lesions have a dry pithy quality suggesting that the tissue was partially dehydrated. Whether the drying of the tissue is a primary factor causing necrosis or merely a change occurring in the cells after they have died, was not determined. It seems likely, however, that the latter situation is the case, since in the external lesions desiccation follows the necrosis of the cells. This point needs further study especially in view of the information that is available through the work of Höfler (1947) and others on different forms of necrosis due to drying of tissue. It should be noted that fruits with very early lesions do not appear wilted, and that the xylem is not an initial site of necrosis. Although by no means conclusive, this suggests that a water deficit is not the primary cause of the disorder.

Calcium Deficiency. The earlier literature on calcium deficiency is reviewed by Sorokin and Sommer (1929), Bamford (1931), Lutman (1934), and others. Kalra (1956) has provided a more recent summary of this field. A number of observations suggest that the symptoms of blossom-end rot are indicative of calcium deficiency.

One typical response to low calcium is the inhibition of elongation of both the root and shoot (Nightingale *et al.* 1931; Fisher, 1935; American Society of Agronomy, 1949; McIlrath, 1950). Under extreme deficiency the root and shoot tips die. In general, calcium deficiency is apparent first in the growing tissues at the extremities of the plant. The fruit, like the root and shoot tips, can be considered to be near the extremities of the plant. Furthermore, the fruit is a site of very active growth particularly when the initial symptoms of blossom-end rot occur.

Walsh and Clarke (1945), McIlrath (1950), and Geraldson *et al.* (1954) have shown that the fruit contains much less calcium than other portions of the plant. McIlrath, for example, reports that in normal plants the percentage of calcium on a dry-weight basis is 2.77, 4.15, 1.76, and 0.40 for the roots, leaves, stems, and fruits respectively. In plants with blossom-end rot Walsh and Clarke (1945), Geraldson *et al.* (1954), and Geraldson (1957*b*) have shown that the calcium content of affected fruit and of the leaves is much lower than normal. Maynard *et al.* (1957) recently reported similar findings for the fruit. Our limited analyses of different portions of the fruit showed that the distal portion of the fruit is much lower in calcium content than the basal portion. Although these analyses were on mature fruit, it appears likely that the calcium content in the blossom-end of rapidly developing fruit may become deficient, with the result that the rot develops.

Gustafson (1927*b*) followed the changes in chemical composition of tomato fruits during their growth. About three weeks after anthesis the calcium content begins to increase considerably. This suggests that the initial symptoms of blossom-end rot may not occur in fruits of advanced stages of growth because of their higher calcium content. The fact that the fruit develops blossom-end rot, yet the leaves and shoot tips of the same plant do not show any low-calcium symptoms, may seem to be an anomaly. This can probably be accounted for by the striking difference in calcium content between the leaves and the fruit. In the experiments of Walsh and Clarke (1945), for example, the range of calcium (percentage of dry weight) for the fruit was 0.04 to 0.24, whereas the range for the upper leaves was 0.64 to 2.94. Since the fruit is relatively low in calcium content, it may be especially vulnerable to changes in calcium nutrition. Venning's (1954) findings on castor bean are of interest in this regard. He concluded that the effects of calcium deficiency are much more profound when the plant is in the flowering and fruiting stage than when in vegetative growth. A similar situation occurs in the peanut. Colwell and Brady (1945) reported that although very low levels of soil calcium are adequate for vegetative growth, a relatively large supply is necessary for proper development of the fruit. Brady (1947) also commented on the fact that an abundance of calcium may occur in the leaves and stems of the peanut, yet the supply to the fruit may be so low as to cause almost complete fruit failure.

One of the responses to calcium deficiency is the accumulation of carbohydrates in the plant. Boehm (1875) observed abnormal accumulations of starch in calcium-deficient beans and Schimper (1890) reported similar findings for the leaves of *Tradescantia.* Venning (1954) found large quantities of glucose and starch in calcium-deficient castor bean stems. Similar

findings have been reported for the tomato. In studies on blossom-end rot Brooks (1914) reported that the cells of the affected tissue were nearly always gorged with starch. Nightingale *et al.* (1931) showed that enormous concentrations of sugar and starch occurred in calcium-deficient tomato plants. McIlrath (1950) found that the percentage of dry weight in calcium-deficient tomato fruits was much higher than in the controls. Lyon and Garcia (1944) suggest that calcium has an indirect influence on the utilization of carbohydrates and nitrogenous food materials in the tomato. My observations confirm those of Smith (1935) and Michailowa (1935) that starch grains may be abundant in the pericarp of the normal tomato. It is evident, however, that starch grains often occur in exceptionally large amounts in tissue affected by blossom-end rot. Although observations of this type were not on a quantitative basis, it appears that this is typical of fruits affected by blossom-end rot. It is accordingly interpreted as a symptom of calcium deficiency in the fruit. This, of course, is not an absolute criterion of calcium deficiency since other disorders, such as boron deficiency, are known to cause accumulations of starch (Gauch and Dugger, 1954).

A number of workers have reported that under calcium deficiency mitosis is aberrant or suppressed or that nuclear structure and division is strongly affected (Reed, 1907; Sorokin and Sommer, 1929, 1940; Davis, 1949; Fink, 1950; Venning, 1953). The effect on mitosis is difficult to evaluate in the material used in this study. When the fruit enters the period of ontogeny in which it may show symptoms of blossom-end rot, its development is characterized by cell enlargement. In this regard Houghtaling (1935) and Gustafson and Houghtaling (1939) found that little or no cell division occurs after anthesis in the ovary wall. Smith (1935), in a less detailed study of this aspect of development, reports that cell division continues until about 14 days after pollination. The rather striking wounding response of the tissues adjacent to the blossom-end rot lesions shows that the cells are capable of division. Yet, the necrosis of some of the cells that have undergone division indicates that some abnormality—perhaps calcium deficiency—affects the newly formed tissue. Schreven (1939) reported that in calcium-deficient potato tubers the tissue adjoining groups of necrotic cells shows numerous cell divisions parallel to the dead tissue. He mentions that this is similar to a normal wound reaction. His figures 11C and 11D suggest that some of the cells that divide in response to the necrosis die as the lesion becomes larger. Although this may not be a diagnostic feature for calcium deficiency, it is of interest that a similar condition often occurs in blossom-end rot tomatoes.

In addition to its effect on cell division, calcium deficiency also causes changes in the cytoplasm. Bamford (1931) describes the effects on wheat roots as a gradual erosion of the cytoplasm until it completely disappears. Sorokin and Sommer (1940) report that in newly formed lateral roots of peas grown under calcium deficiency the cytoplasm decreases in amount. Florell (1956) has shown by chemical methods that in wheat roots the mitochondrial fraction as well as its protein content is much less under calcium deficiency than under adequate calcium nutrition. He suggests that calcium favors the formation of mitochondria by means of its general influence on the organization of the cytoplasm. In tomato fruits affected by blossom-end

rot the cytoplasm undergoes marked changes, but there is no obvious reduction in the visible particulate components. The most striking change in some necrotic cells of blossom-end rot fruit is the development of light-yellow to brown granules or inclusions in the cytoplasm. This change seems to be indicative of proteinaceous degradation because the granules give a positive test for proteins. Nightingale *et al.* (1931) reported the presence of golden-brown proteinaceous inclusions in the necrotic cells of calcium-deficient tomato fruits. Brooks (1914) also described the presence of granules of congested protoplasm in blossom-end rot fruits, but he did not mention their color or test their composition. Although granules often occur in degenerated cytoplasm (Küster, 1925), the granules or inclusions observed in this study and by Brooks (1914) and Nightingale *et al.* (1931) may be characteristic of calcium-deficient tomatoes. Similar observations have been made in the potato. Bolle-Jones (1955) reported that in calcium-deficient tubers the necrotic cells contain numerous orange-brown granules. Reddish-brown discoloration due to calcium deficiency has been observed in wheat roots (Florell, 1956) and in strawberry and sugar cane (Kitchen, 1948). It would be of interest to know the cytological aspects of the discoloration in these plants.

SUMMARY

An account of the early research on blossom-end rot is given and a large body of evidence is reviewed from the more recent horticultural and physiological literature implicating calcium deficiency in the fruit as the fundamental cause of the disorder.

Flowers of field-grown San Marzano tomatoes were tagged at anthesis to provide fruits of known age which were later collected and observed for blossom-end rot and to determine the relationship of the disorder to fruit growth.

Incipient stages of the rot occurred about 10 to 15 days after anthesis when the fruit was about 2.7 to 4.2 cm long or from 38 per cent to 60 per cent full grown on the basis of length. During this phase of fruit development the increments of growth in length are very large in relation to the size of the fruit. The inception of the disorder is associated with an active phase of fruit development.

Both externally visible and deep-seated symptoms occur separately or together in the same fruit at the distal end. In general, the symptoms involve a progressive necrosis of groups of cells with loss of water from them. The developing lesions eventually involve, in many cases, the entire distal end of the fruit and the affected fruits are retarded in length growth.

Cytologically, the first indication of the externally visible lesions is the development of brown proteinaceous inclusions in the epidermis and in the underlying cells of the pericarp. The necrotic cells may collapse and a wound healing response is usually evoked in the adjacent living tissue. Cells arising by this process may in turn become necrotic.

Light-yellow to brown proteinaceous inclusions also develop in the deep-seated lesions, particularly in parenchyma cells associated with the vascular bundles of the placental axis. Degradation products in some cells show a typical wound gum staining reaction and positive tests for proteins.

Although normal young fruits contain starch, the cells of affected fruits are often glutted with starch grains.

Cells affected by blossom-end rot appear to become dehydrated to some extent after necrosis, but no conclusive histological evidence could be found to support the view that the disorder is primarily due to water stress.

A number of lines of evidence presented here, with supporting evidence from the literature, implicate calcium deficiency in the fruit as the basic cause of blossom-end rot in the tomato:

(1) This study confirms that the fruit is relatively low in calcium content. According to the literature the mature leaves are high and young fruits are especially low in calcium.

(2) It is widely held in the literature that the calcium in the mature portions of the plant is essentially not available to the growing parts. Accordingly, the first symptoms of calcium deficiency, the inhibition of elongation, occur in the growing extremities of plants. From calcium analyses of different portions of the fruit made in this study it is inferred that the distal ends of young elongated fruits are relatively low in calcium and therefore subject to calcium deficiency.

(3) Carbohydrates, starch as observed in this study, are reported to be excessively high in calcium-deficient plants.

(4) Recently divided cells in wound meristems associated with the lesions in the tomato fruit undergo necrosis, a condition also reported in other plants affected by calcium deficiency.

(5) The protoplasm shows evidence of calcium deficiency through the formation of light-yellow to brown proteinaceous inclusions, a feature also reported for calcium deficiency in some plants.

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