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HISTOLOGICAL CHARACTERISTICS OF PLANTS GROWN IN TOXIC CONCENTRATIONS OF BORON[1]

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INTRODUCTION

Although small amounts of boron are considered essential for the normal growth and development of many widely different species of the higher green plants (*7, 8, 15, 16, 17*),[2] excessive concentrations of this element have long been known to be toxic (*11*). Injury resulting from boron occurring in excessive concentrations as a natural constituent of irrigation waters is a factor of agricultural importance in certain areas in tne western United States (*5, 9, 13*). The wide variability of optimum and maximum concentrations of boron for the growth of different species and the characteristic external symptoms of boron injury exhibited by numerous plants have been discussed recently by Eaton (*5*). Generally, leaf injury is the characteristic manifestation of boron toxicity, the older leaves showing the most pronounced symptoms. Briefly, the common symptoms of boron injury to leaves are the yellowing of tips and margins (often followed by marginal or spotted browning, which may be followed by premature defoliation) and the subsequent production of malformed leaves. Under high-boron conditions stone-fruit trees seldom exhibit these leaf symptoms, but the petioles and larger veins of the leaves of prune and apricot trees may become brown and rough and occasionally exude gum. The twigs frequently exude gum, especially above the leaf and twig insertions, and die at the tips; their internodes may be much shortened, and their nodes are often enlarged.

MATERIAL AND METHODS

The histology of plant organs showing boron injury has hitherto received but scant attention. The specimens upon which the present study is based were mostly taken from the cultures of this Division at the Rubidoux Laboratory, Riverside, Calif. The boron-injured prune specimens were from a plant grown in a sand culture supplied with a culture solution containing 9 parts per million of boron. The boron-injured peach, apricot, and grape were supplied with a solution containing 6 p. p. m. of boron; and the boron-injured lemon was supplied with a solution containing 3 p. p. m. of boron. These specimens were studied in comparison with healthy specimens of the same age and variety grown under similar conditions except for lower concentrations of boron in the culture solution. In the case of lemon and grape, the healthy specimens received 1 p. p. m. of boron in the culture solution. Specimens of healthy peach, prune, and apricot

[1] Received for publication Nov. 12, 1934; issued April 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 193.

were grown in a culture solution to which no boron had been added. The boron contained in these solutions was generally less than 0.1 p. p. m., occurring as impurities in the c. p. chemicals or in the quartz sand. Specimens of field-grown prunes and apricots supplied with irrigation water of high boron content and comparable specimens from adjacent orchards irrigated with water of low boron content were also examined. The material was sectioned fresh or after killing and fixing in formalin-acetic-alcohol, and mounted unstained in glycerin.

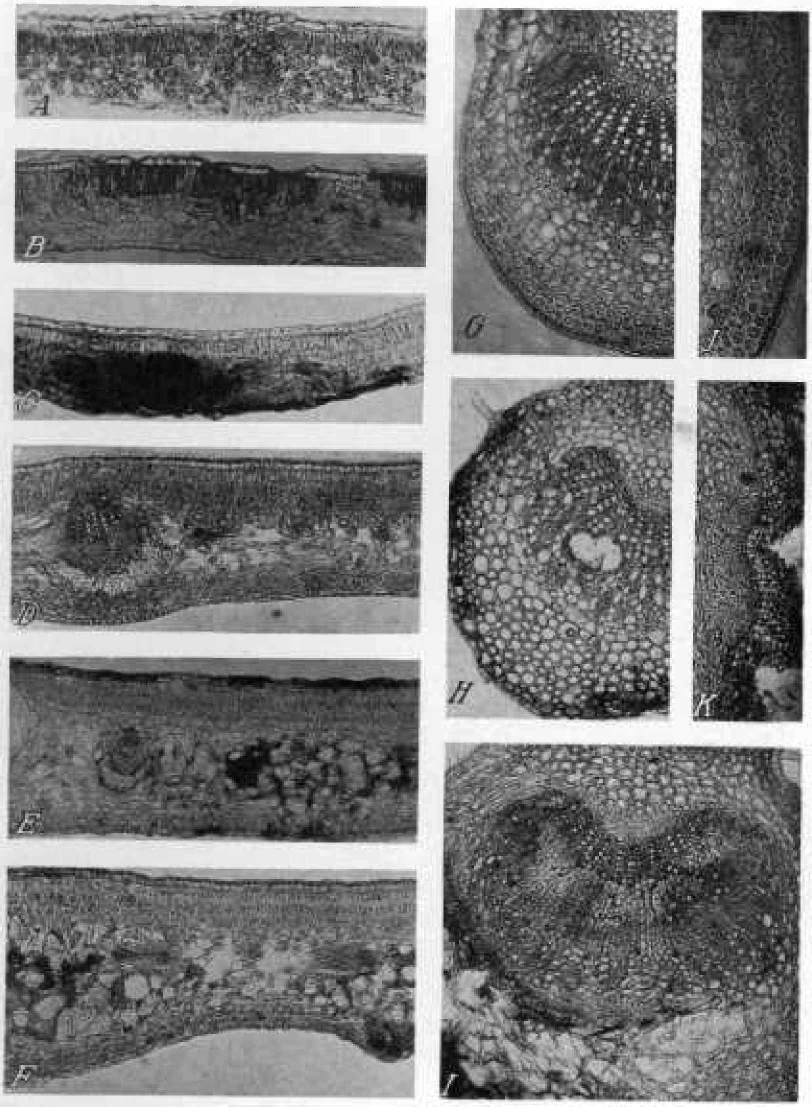
ABNORMALITIES OF LEAVES

A histological study of boron-injured leaves of lemon, grape, prune, and apricot showed that their structural abnormalities occur chiefly at points showing macroscopically visible injury. The yellowish areas which commonly precede the brown margins of boron-injured lemon leaves and the pale-green areas which often border the brown spots of boron-injured grape leaves are attributable to the conversion of chloroplasts into leucoplasts in such areas. Except for the reduced number of chloroplasts, the mesophyll of injured leaves shows no histological differences from that of healthy leaves at these points (pl. 1, *A–F*).

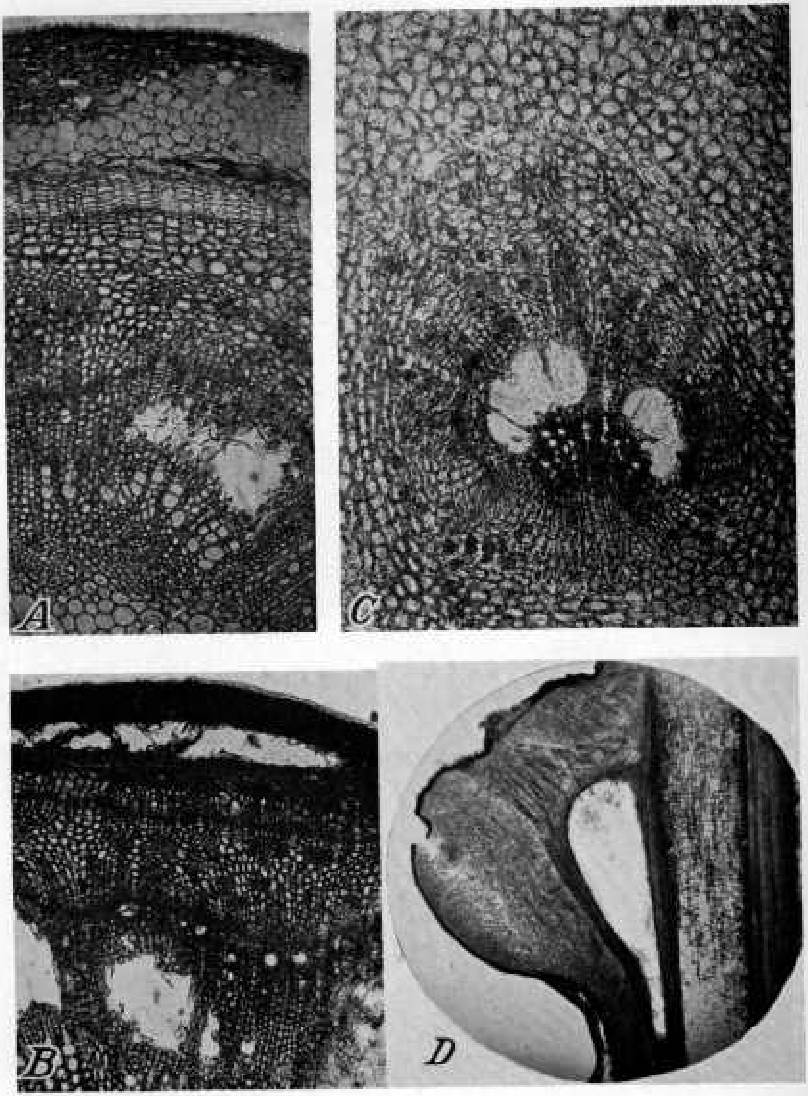
Cells of normal size and arrangement, showing various stages in the disorganization of their protoplasts, are visible in the discolored areas present at or near the margins of boron-injured lemon and grape leaves and in the larger veins and petioles of boron-affected grape, apricot, and prune leaves (pl. 1, *B*, *C*, *E*, *H*). In the early stages of this protoplasmic disintegration, the browned protoplasts completely fill the cell lumina, but later they often shrink away from the cell walls. Such browning of cells is at times observable in the epidermis, palisade parenchyma, spongy parenchyma, and parenchyma of the larger veins, but usually does not extend from upper epidermis to lower epidermis at a given point in the lamina. The conducting tissues of veinlets surrounded by necrotic mesophyll are frequently filled with brownish contents of gummy appearance.

Associated with such necrotic areas, very limited hypertrophied and hyperplastic areas are occasionally visible in the mesophyll of boron-injured lemon leaves (pl. 1, *F*) and in the petioles and midribs of boron-injured prune (pl. 1, *I*) and apricot leaves. At times a phellogen layer develops beneath the necrotic parenchyma of apricot and prune petioles (pl. 1, *K*) and midribs, resulting in the sloughing off of small, macroscopically visible scales of dead tissue. In prune petioles and midribs with externally visible injuries, gum pockets formed by disintegration of xylem elements may be present (pl. 1, *H*). The absence of changes in the vascular anatomy of boron-injured citrus leaves is reported by Haas (*6*).

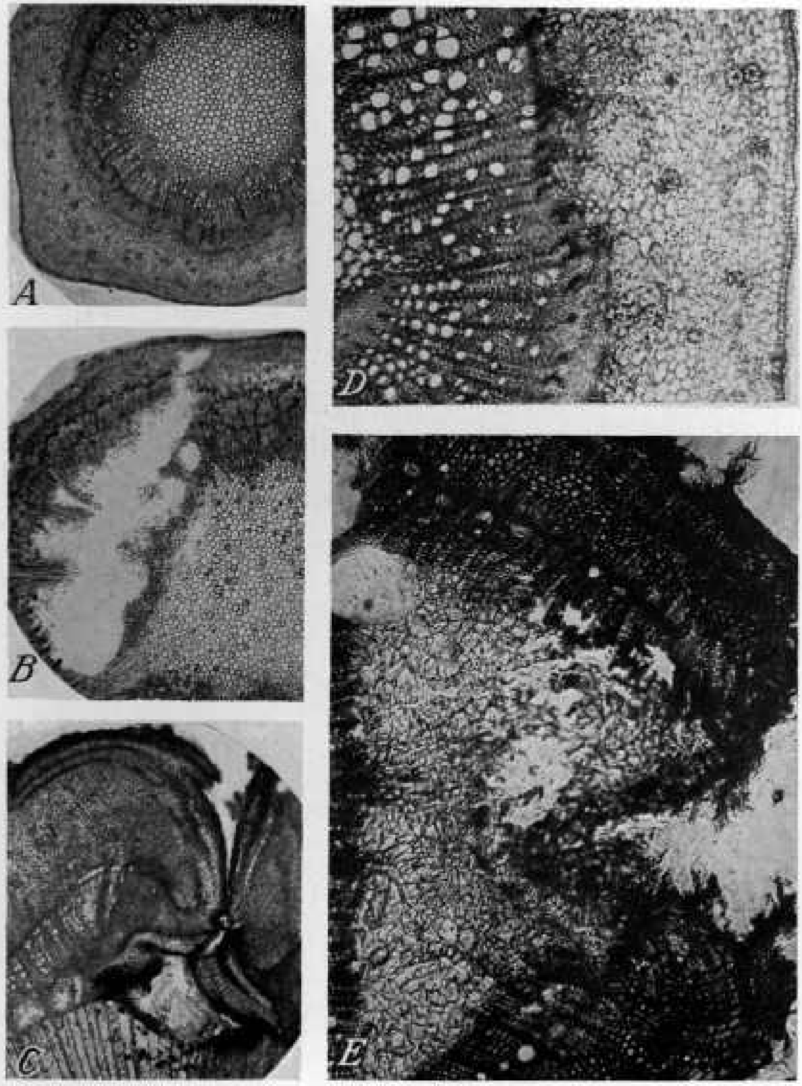
The only additional way in which boron-injured leaves were observed to differ histologically from comparable healthy leaves is in the presence of fewer calcium oxalate crystals in the injured leaves. This difference is most noticeable in the phloem of petioles and veins of apricot and prune leaves (pl. 1, *G*, *H*). In this connection it is noteworthy that Haas (*6*) reports that chemical analyses have shown that boron-injured lemon and walnut leaves contain a lower percentage of calcium and a higher percentage of potassium than healthy leaves of these species.



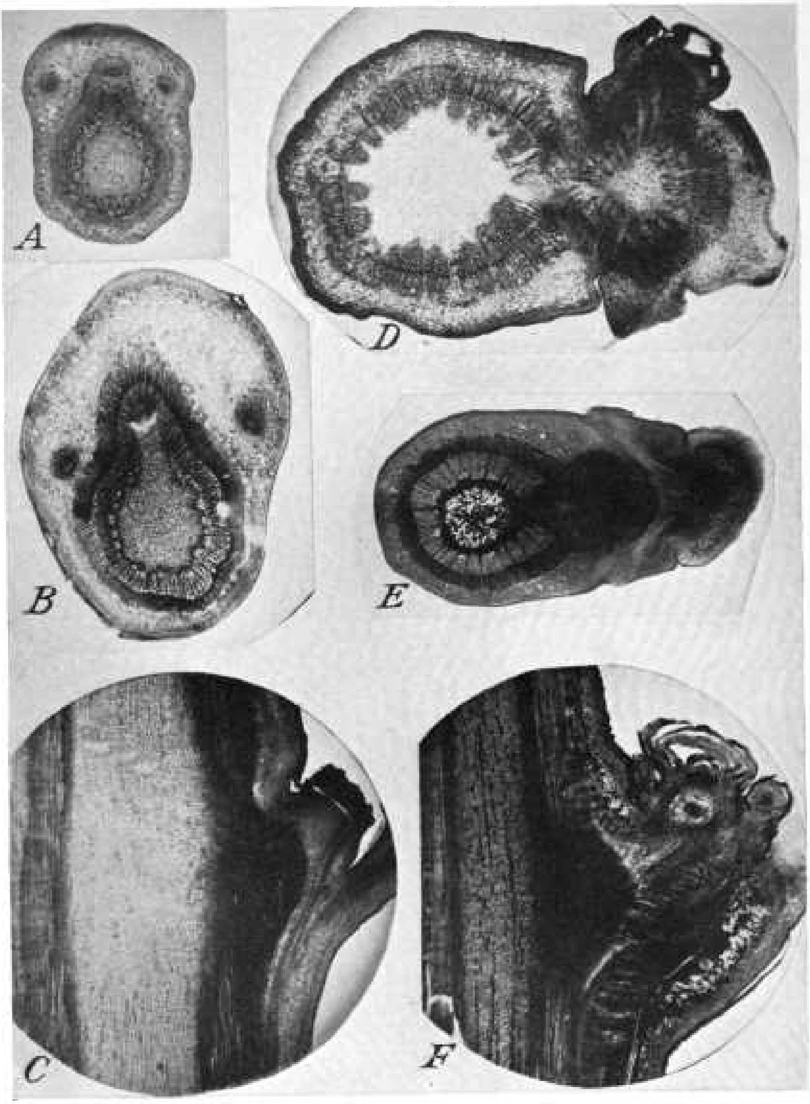
A–F, Cross sections of leaf blades: *A*, Healthy grape; *B–C*, boron-injured grape; *D*, healthy lemon; *E–F*, boron-injured lemon. × 50. *G–I*, Cross sections of prune midribs: *G*, Healthy; *H–I*, boron-injured. × 35. *J–K*, Cross sections of prune petioles: *J*, Healthy; *K*, boron-injured. × 35.



A–B, Cross sections of internodes of current season's stems: *A*, Boron-injured prune; *B*, boron-injured peach. × 79. *C*, Cross section of brauch trace in node of boron-injured prune. × 79. *D*, Longitudinal section of node of boron-injured prune. × 9.



A–B, Cross sections of internodes of current season's stems of apricot: *A*, Healthy; *B*, boron-injured × 9. *C*, Cross section of internode of 2-year-old stem of boron-injured prune. × 9. *D–E*, Cross sections of internodes of current season's stems of peach: *D*, Healthy; *E*, horon-injured. × 79.



A–B, Cross sections of nodes of current season's stems of apricot: *A*, Healthy; *B*, boron-injured. × 7.5. *C*, Longitudinal section of node of healthy apricot. × 7.5. *D–E*, Cross sections of nodes of current season's stems of prunes: *D*, Boron-injured; *E*, healthy. × 7.5. *F*, Longitudinal section of node of boron-injured apricot. × 7.5.

ABNORMALITIES OF STEMS

The greatest abnormalities in stem structure of boron-injured stone-fruit trees are to be found in shoots that do not live beyond the first year, although enlarged nodes are often conspicuous on 2- and 3-year-old branches. In current season's stems of boron-affected prune, peach, and apricot trees, necrotic areas comparable to those discussed in connection with boron-injured leaves occur in the epidermis and subjacent cortical parenchyma (pl. 2, *A*). As in the case of petioles of these species, phellogen (pl. 2, *A*) may develop beneath the necrotic tissues which at times slough off in the form of small scales. Since the necrotic areas of cortical parenchyma are commonly lenticular in cross section and may extend deeply into the cortex, such phellogen formation is in contrast to that normally occurring in the subepidermal layer of cortical parenchyma cells. As in the leaves, the current season's stems of boron-injured stone-fruit trees, particularly those of peach and apricot, may contain noticeably fewer calcium oxalate crystals than comparable healthy specimens (pl. 3, *A*, *B*, *D*, *E*). This difference in calcium oxalate content is visible in cortex, phloem, and pith.

Small lysigenous cavities filled with gum are frequently present in the cortex of first-year stems of boron-injured stone-fruit trees (pl. 2, *B;* pl. 3, *B*). The pressure exerted by the gum in these cavities is often sufficient to rupture the epidermis, but at times merely distends the overlying tissues and causes minute swellings on the periphery of the stem. Although such cortical gum cavities are rather numerous, axial lysigenous gum ducts in the xylem are far more abundant and conspicuous (pl. 2; pl. 3, *B*, *C*, *E*). The gum ducts are apparently formed by the disintegration of abnormal xylem parenchyma. Such tissue at times persists and may form a large percentage of the wood normally composed of other axially elongated xylem elements (pl. 3, *E*). In the internodes the gum ducts in the xylem may encircle the stem. At the nodes, xylem elements of leaf and branch traces, as well as those of the main vascular cylinder, may disintegrate to form gum ducts (pl. 2, *C*). Occasionally cortical gum cavities and gum ducts in the xylem become united through the disintegration of intervening cambium, phloem, and cortical elements (pl. 3, *B*, *C*). Such fusion of gum cavities indicates that a large proportion of the gum which accumulates on the surface of these stems has its origin in the xylem. Gum pockets may also occur in the pith immediately beneath the dead portion of twigs which have died at the tips. Gum ducts similar to those occurring in first-year stems may also be formed in later years (pl. 3, *C*).

The enlargement of nodes of boron-injured stone-fruit trees (pl. 2, *D;* pl. 4, *B*, *D*, *F*) is chiefly due to the growth of axillary and accessory buds which would normally remain dormant (pl. 4, *C*–*F*). The branch traces of such nodes are therefore usually considerably larger than those in healthy stems. More or less hyperplasia commonly occurs in the cortex of the enlarged nodes (pl. 2, *D;* pl. 4, *A*, *B*), and hypertrophy of some of the parenchyma cells is not unusual. In the shortened internodes of boron-injured stone-fruit stems the cortical parenchyma cells remain shorter and exhibit less variation in size than similar cells in comparable healthy stems.

DISCUSSION

From the foregoing it is evident that the manifestations of boron toxicity which are characteristic of some plants are lacking in others. Butler (*2*) has cited numerous instances in which similar reactions of tissues may be brought about by widely different stimuli. Hence it is not surprising to find that the histological evidences of injury by boron are, in general, similar to those attributable to other causes. The enlarged nodes of boron-injured stone-fruit trees are not unlike those of French prune twigs affected with exanthema (*14*). Lysigenous gum ducts in stone-fruit trees have been induced by a wide variety of stimuli including a number of fungi, bacteria, insects, chemical substances, and traumatisms (*3*). Priestley and Woffenden (*12*) have shown that phellogen formation in both normal and abnormal positions results from a set of conditions that may be fulfilled in many ways. The calcium oxalate content of the aboveground organs of plants is influenced by various factors which influence the rate of transpiration (*10*), as well as by the composition of the nutrient solution (*1*). Hypertrophied cells and hyperplastic areas in leaves may result from such diversified stimuli as fungi, insects, injurious vapors (*2*), and boron deficiency (*7*). Chloroplasts frequently change back into leucoplasts in starving cells and in those incited to abnormal growth (*10*). Localized browning of cells may be initiated by sunburn (*4*), wounding (*10*), or virus infection.

The cases cited above are sufficient to indicate that a toxic concentration of boron in the nutrient solution cannot be regarded as a stimulus to a specific reaction reflected as a specific histological change, but that the histological changes induced by excessive concentrations of boron are correlated with the inherent capacity of a species to respond to stimuli. Theoretically every living plant cell possesses the potential capacity to react to various stimuli by hypertrophy, hyperplasy, or the development of meristematic tissues (*2*, *10*), but the potentiality is greater in certain tissues than in others, and these tissues show greater potentiality in certain species than in others. It has been suggested that one of the roles which boron plays in the plant is that of stimulating meristematic activity (*1*, *7*, *8*, *16*, *17*). Structural abnormalities resulting from stimulation to abnormal growth are met with in the stems, petioles, and veins of some boron-injured stone-fruit trees and to a very limited extent in boron-injured lemon leaves. Such of these structural abnormalities as occur in the cortex of stems, the parenchyma of petioles and veins, and the mesophyll of leaves are commonly associated with necrotic areas in which the cells are of normal size and arrangement. Similar necrotic areas are found in the mesophyll of boron-injured lemon and grape leaves which show no structural abnormalities. These necrotic areas are commonly preceded and bordered by areas in which partial degeneration of chloroplasts indicates a weakened condition of the protoplast.

Kelley and Brown (*9*) and subsequently Scofield and Wilcox (*13*) have shown that boron accumulates in the leaves of boron-injured citrus and walnuts. Eaton (*5*) has shown further that the tissues manifesting the greatest injury are those in which boron has accumulated in relatively high concentrations. It seems probable, therefore, that an excessive concentration of boron in the cell often injures the protoplast to such an extent that it undergoes a progressive degeneration first affecting the chloroplasts. However, in some instances an excess of boron may stimulate the protoplast to abnormal growth or division.

SUMMARY

Injury to plants resulting from boron occurring in excessive concentrations as a natural constituent of irrigation waters is a factor of agricultural importance in certain areas in the western United States.

The macroscopic symptoms of boron injury to various plants have been described in previous papers.

Histological characteristics of boron-injured lemon, grape, prune, and apricot leaves, and prune, peach, and apricot stems are described herein.

In general, the plant parts manifesting the greatest injury are those in which boron has accumulated in relatively high concentrations.

Both macroscopic and microscopic manifestations of boron toxicity which are characteristic of some plants are lacking in others. This indicates that a toxic concentration of boron in the nutrient solution is not a stimulus to a specific reaction reflected as a specific histological change.

The histological evidences of injury by boron are, in general, similar to those attributable to other causes. This suggests that the observed abnormalities are correlated with the inherent capacity of a species to respond to stimuli.

It seems probable that an excessive concentration of boron in the cell often injures the protoplast to such an extent that it undergoes a progressive degeneration. Chloroplasts, when present, are affected first.

In some instances an excess of boron may stimulate the protoplast to abnormal growth or division.

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