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KATHERINE ESAU

UNIVERSITY OF CALIFORNIA · BERKELEY, CALIFORNIA

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Virus of curly top disease was introduced, through one cotyledon, into seedlings of a highly susceptible sugar beet strain. The inoculated cotyledon developed phloem hyperplasia—abnormal multiplication of cells—characteristic of the curly top disease. This symptom was observed in the main vein and some small veins on the third day after inoculation. In many samples, the main vascular bundle also showed pronounced necrotic obliteration in the older part of the phloem tissue, a symptom not previously described for curly top infection.

Noninoculated cotyledons collected twelve days after inoculation of the experimental seedlings had developed no curly top symptoms.

ANATOMIC EFFECTS OF BARLEY YELLOW DWARF VIRUS AND MALEIC HYDRAZIDE ON CERTAIN GRAMINEAE

Infection with the virus of barley yellow dwarf disease and treatment with maleic hydrazide produce many similar external and internal degenerative changes. These are depression of growth, the yellowing of leaves, the forming of sugary exudate on leaves, and the necrosis of certain tissues, particularly of the phloem.

A significant difference exists between the initial effect of the virus and that of maleic hydrazide in the growing parts of shoot and root. Virus injury is first recognized, anatomically, in the young phloem, whereas the first effect of maleic hydrazide is expressed in the inhibition of meristematic activity. The initial derangement in the phloem induced by the virus is of such a nature that the existence of a close relation between the virus and the phloem may be inferred: the virus seems to move in the mature sieve elements. Maleic hydrazide does not appear to be primarily associated with the phloem, but it also probably enters this tissue (the phloem) since in older phloem it induces degenerative changes similar to those found in the yellow dwarf plants.

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KATHERINE ESAU[3]

INTRODUCTION

As IS CHARACTERISTIC of virus diseases, the external symptoms of curly top usually develop on leaves that are younger than the one through which the plant was inoculated (Esau, 1935, 1941).[4] The internal symptoms also are most pronounced in young parts of the plant, but they may develop in the inoculated leaf even if the latter shows no external symptoms. In certain studies on the spread of curly top symptoms the inoculated leaf was a cotyledon, and in some collections it, too, developed phloem abnormalities (Esau, 1935). Further work on the development of internal symptoms in the inoculated cotyledon was initiated in connection with the studies mentioned above, but the material was only recently processed for microscopic study. Observations on this material are reported in the present paper.

MATERIAL AND METHODS

Sugar beet seedlings of a strain recorded under the number 2769 by the United States Department of Agriculture—a strain highly susceptible to curly top—were inoculated through one of the cotyledons when the first two foliage leaves were approximately 0.5 cm long. The inoculations were made by allowing one viruliferous beet leafhopper (*Circulifer tennelus*) to feed upon the upper half of the cotyledon for twenty-four hours. This cotyledon and the shoot parts above it were then collected for study. The collections were repeated on five successive days. Additional collections were made from plants that had been inoculated earlier in connection with some other experiments. These additional collections consisted of inoculated cotyledons from plants inoculated seven days before the sampling and of inoculated and noninoculated cotyledons from plants inoculated twelve days before the sampling. Cotyledons of various ages were collected from noninoculated seedlings for the controls.

An ordinary paraffin method was employed for the preparation of slides. Hematoxylin and safranin were used for staining.

[1] Received for publication February 25, 1957.

[2] All work was carried out in a greenhouse at Riverside, California, in coöperation with Dr. C. W. Bennett of the United States Department of Agriculture.

[3] Professor of Botany and Botanist in the Experiment Station, Davis.

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

OBSERVATIONS

The Phloem of the Sugar Beet. Since the phloem of the sugar beet has been described in several articles (e.g., Artschwager, 1926; Esau, 1933), a brief summary of the information will suffice here. Figure 1 shows sections from a large (*A*) and a small (*B*) vein from a petiole of a foliage leaf. The bundles of two different sizes show the difference in structure between the phloem of a vein that begins to mature before the leaf completes its expansion (large bundle, fig. 1,*A*) and that of a vein maturing later (small bundle, fig. 1,*B*). In a large vein, the early part of the phloem (protophloem) is subjected to stresses resulting from the growth of surrounding tissues, and its sieve elements and companion cells are crushed and obliterated. In the small veins, no such crushing occurs.

Figure 1,*A*, at left, shows the old phloem in which the results of crushing of sieve elements are recognizable as thick areas (*e*) among large cells (*c*). These are the parenchyma cells of the old phloem, now much enlarged and having thickened walls. To the right of this tissue—sometimes referred to as the "bundle cap"—is the active phloem (metaphloem) in which the sieve elements (*a*) are accompanied by densely stained companion cells. The phloem-parenchyma cells (*c*) are here only somewhat wider than the sieve elements. To the right of the phloem is the vascular meristem. It was active when the leaf was sampled as is evidenced by the occurrence of partly differentiated sieve elements (*b*) and xylem elements (*d*) along its margins.

Figure 1,*B* shows that the phloem of a small bundle contains the same elements as that of the large: sieve elements (*a*) with companion cells and phloem-parenchyma cells (*c*). A few immature cells occur between the phloem and the xylem (*d*).

Phloem Degeneration Above the Site of Inoculation. Precise information on the anatomic changes in curly top-infected plants refers mainly to symptoms that develop in plant parts to which the virus moves, from the site of inoculation, in its systemic spread. Therefore, development of symptoms in the foliage leaves above the cotyledons should be examined for comparison with symptoms in the cotyledon into which the virus is introduced directly by the insect. It has been shown previously (Esau, 1935, 1941) that curly top symptoms develop in young plant parts in spatial relation to mature sieve elements. Cells in the vicinity of such elements undergo growth changes, notably hyperplasia. Figures 2 and 3 compare vascular bundles from the first foliage leaves before (fig. 2) and after (fig. 3) the histologic symptoms began to develop. In figure 2,*A*, several mature sieve elements (*a*) are present. One (at *b*) appears in an early stage of normal obliteration. In the larger bundle in 2,*B*, two or more sieve elements are undergoing obliteration (*b*). The parenchyma cells in the older phloem region are somewhat larger than in the younger tissue. They undergo further enlargement after the obliteration of the sieve elements (cf. fig. 1,*A*).

Figure 3 shows two bundles with some hyperplasia in the phloem. In 3,*A*, cells in two areas of the phloem (*c*) have undergone divisions producing a small-celled, densely cytoplasmic tissue. Normal sieve elements (*a*) are recognizable near and within this tissue. In 3,*B*, hyperplasia has developed in only

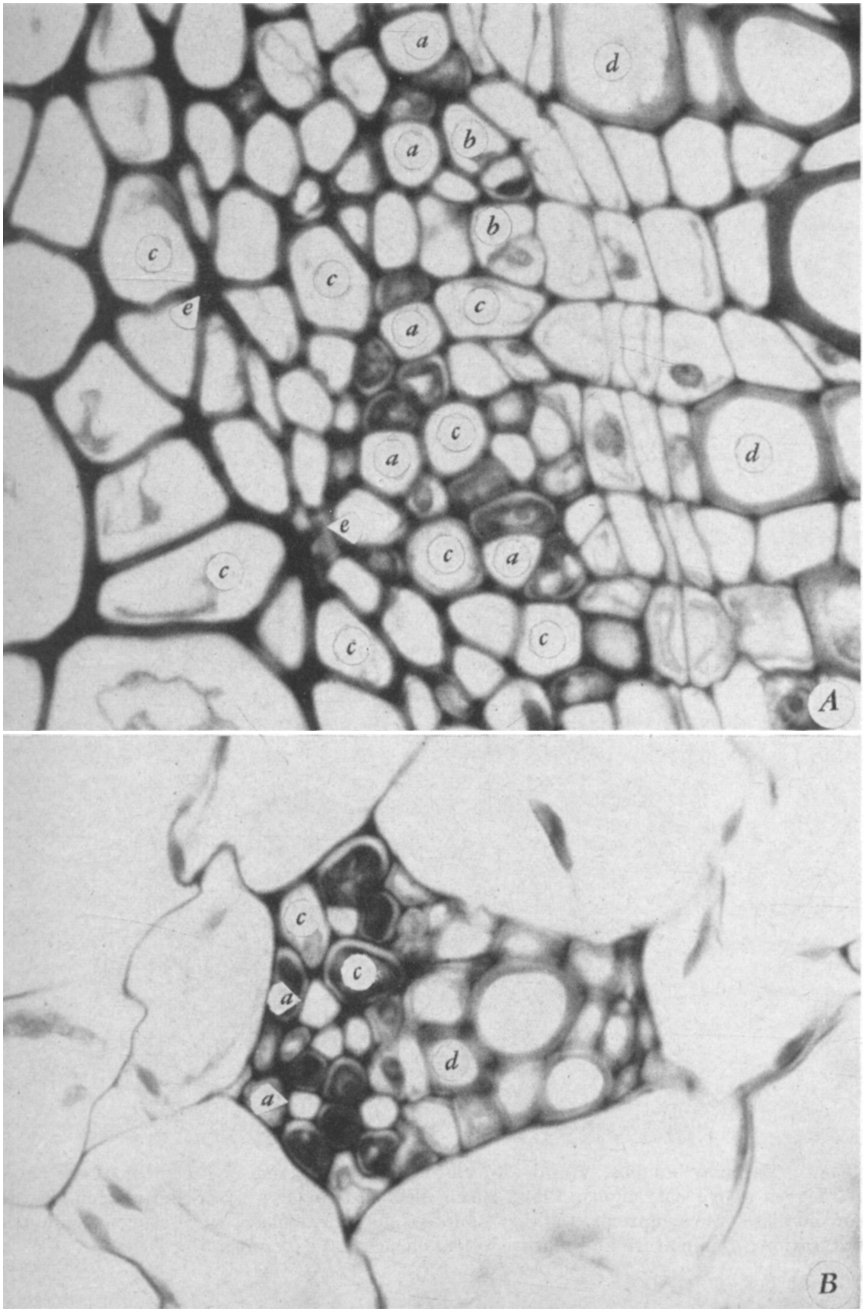

Fig. 1. Phloem of the sugar beet. *A*, Part of large vascular bundle; *B*, small vascular bundle from petiole of a foliage leaf. Details: *a*, mature sieve element; *b*, immature sieve element; *c*, phloem-parenchyma cell; *d*, tracheary element in xylem; *e*, crushed phloem element. (*A*, × 1,100; *B*, × 1,420.)

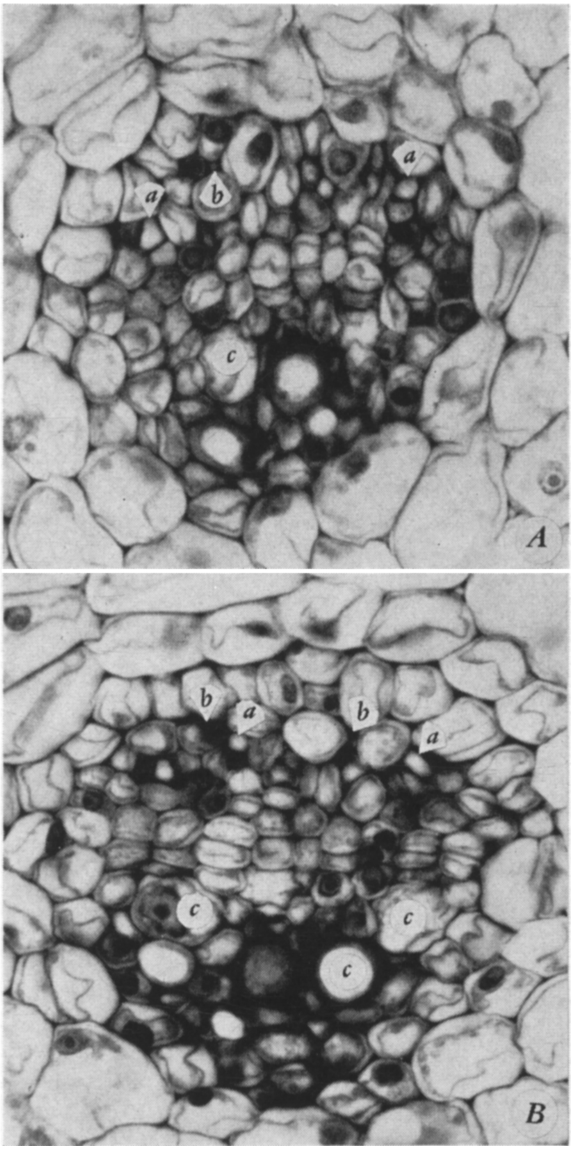

Fig. 2. Vascular bundles, lateral (*A*) and median (*B*), from one member of the pair of first leaves above cotyledons. Plant was collected two days after inoculation. Bundles show no histologic symptoms. Details: *a*, active sieve element; *b*, sieve element undergoing obliteration; *c*, immature and mature xylem elements. (*A*, × 850; *B*, × 760.)

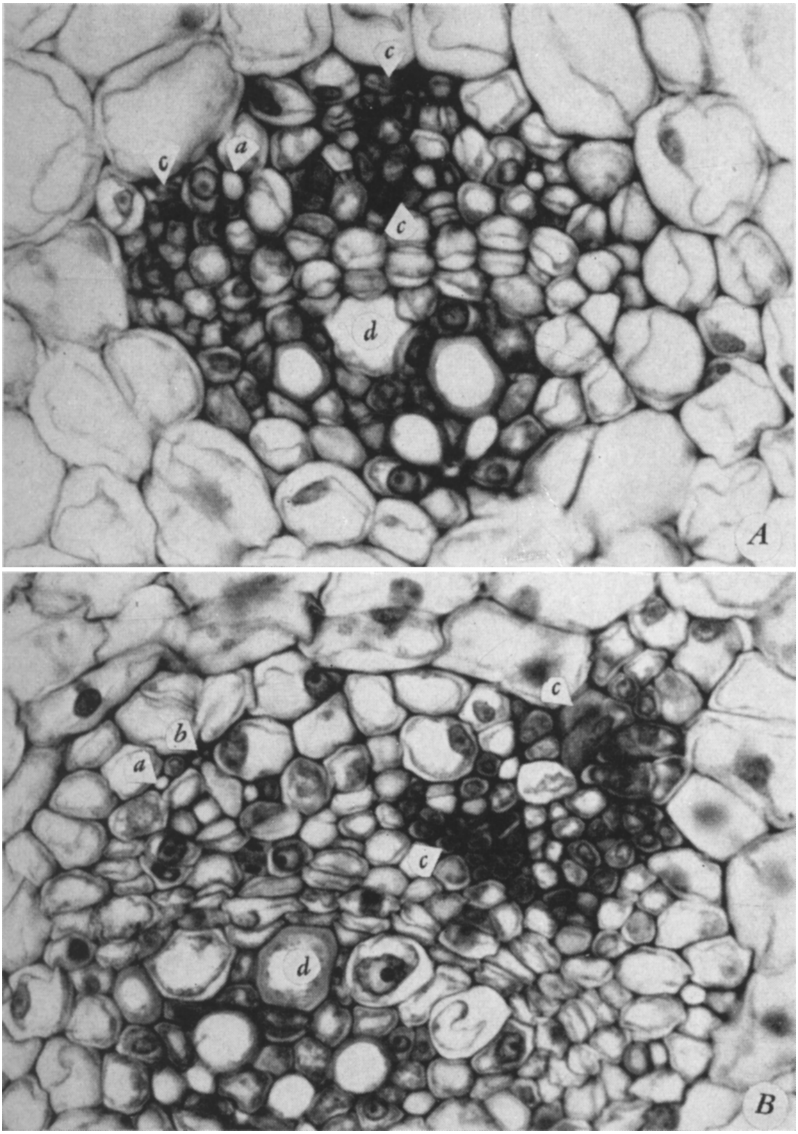

Fig. 3. Vascular bundles, lateral (*A*) and median (*B*), from one member of the pair of first leaves above cotyledons. Plant was collected three days after inoculation. Details: *a*, active sieve element; *b*, sieve element undergoing obliteration; *c*, hyperplastic tissue; *d*, immature xylem element. (*A*, × 850; *B*, × 760.)

one area within the phloem itself, but it has extended also to the parenchyma outside the phloem (above and to the right of *c*). A normally obliterating sieve element appears at *b*. In both bundles the vascular meristem between the phloem and the xylem (*d*) is not yet affected except, possibly, at the left in 3,*A*.

Frequently, hyperplastic divisions are preceded by a hypertrophic enlargement of some of the cells in the immediate neighborhood of mature sieve elements (Esau, 1935, 1941). Furthermore, such cells may undergo necrosis. The young foliage leaves in the present study showed hypertrophy of nuclei in parenchyma cells near the sieve elements, some cell hypertrophy, and, in one leaf of one plant, some severe necrosis near the hyperplastic phloem.

The hyperplastic degeneration of the phloem depicted in figure 3 strikingly contrasts the pathologic changes induced by the curly top virus with those that develop in cereals affected by the yellow dwarf virus of barley (Esau, 1957). In the latter, the phloem symptom is a necrosis and collapse of cells not associated with marked growth changes. Since such a collapse resembles normal obliteration, except that it occurs earlier and involves necrosis of cells, the term "necrotic obliteration" has been adopted for this symptom (Esau, 1957).

Necrotic obliteration is not restricted to plants affected by viruses, but may be induced by various other conditions and agents (e.g., Schneider, 1945, 1954). This symptom seems to be less specific than the hyperplastic changes induced by the curly top and aster yellows (Girolami, 1955) viruses. Although hyperplasia can be induced in the phloem by certain growth-regulating substances (Eames, 1950), the resulting tissue has the structure of parenchyma, whereas, in plants affected by the curly top and aster yellows viruses, the hyperplastic tissue differentiates into a mass of abnormal sieve elements (Esau, 1935, 1941; Girolami, 1955).

Phloem Degeneration in the Inoculated Cotyledon. The cotyledon of the sugar beet has a midvein (see fig. 4,*A*) and a sparse network consisting of small veins of several sizes (see fig. 6).

As has been stressed previously (Esau, 1935, 1941), the typical degenerative changes induced by the curly top virus occur in the active phloem tissue, that is, phloem in which the sieve elements have reached their mature state. If the virus reaches an area where the earliest sieve elements are still active, the oldest part of the phloem is affected (fig. 3). If the earliest sieve elements are past their active stage, the virus affects the younger phloem, whereas the elements of the older phloem undergo an apparently normal obliteration (fig. 3,*B*, at *b*).

Since the cotyledons in the present study were almost fully expanded when they were inoculated, it was to be expected that the oldest phloem would no longer respond to the entry of the virus with hyperplastic changes. The condition of the oldest phloem in the median bundle at the base of the inoculated cotyledon collected two days after the inoculation is shown in figure 4,*A*. The bundle cap of enlarged parenchyma cells appears above, in the area where the earliest sieve elements had become obliterated. Immediately below is the phloem part with sieve elements soon to be obliterated, followed by the

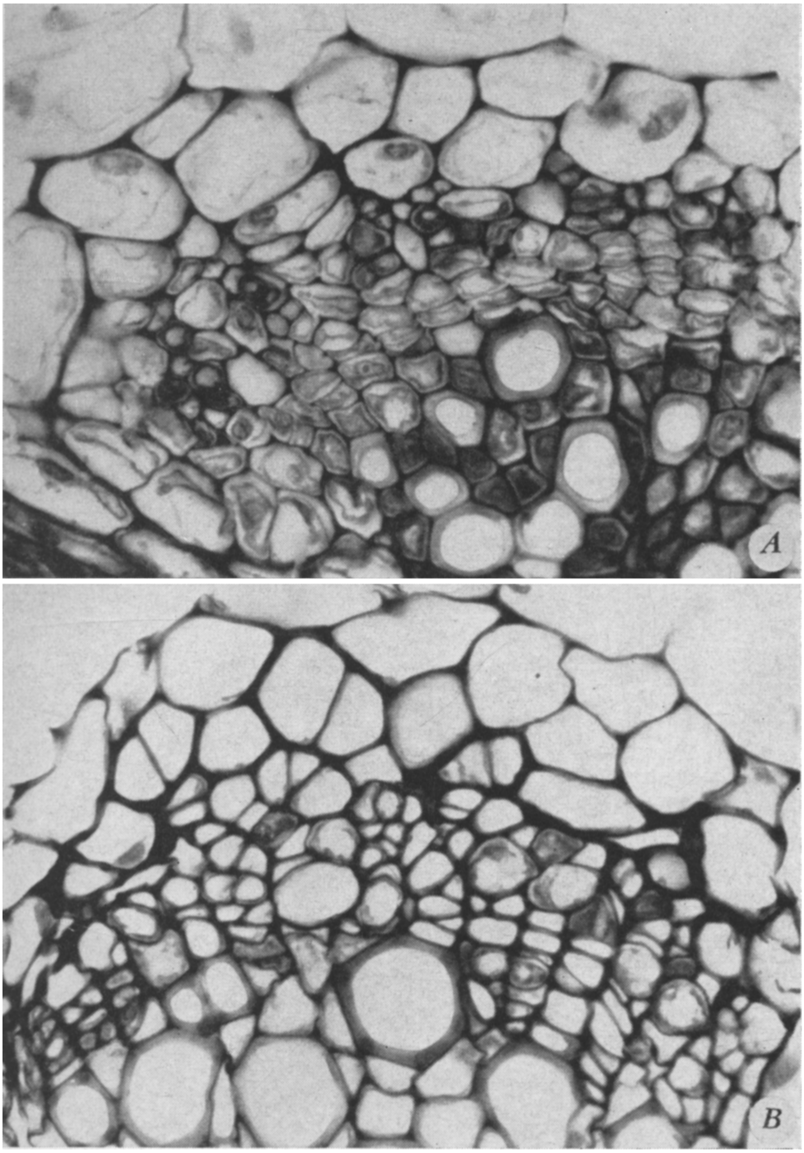

Fig. 4. Median vascular bundles from basal parts of inoculated cotyledons. *A*, Bundle without histologic symptoms, from a plant collected two days after inoculation; *B*, bundle with advanced hyperplasia and some necrotic obliteration (dark stain) from a plant collected twelve days after inoculation. A comparison of *B* with *A* and with fig. 1,*A* shows that the meristem between xylem and phloem in *B* has differentiated into phloem. (Both × 760.)

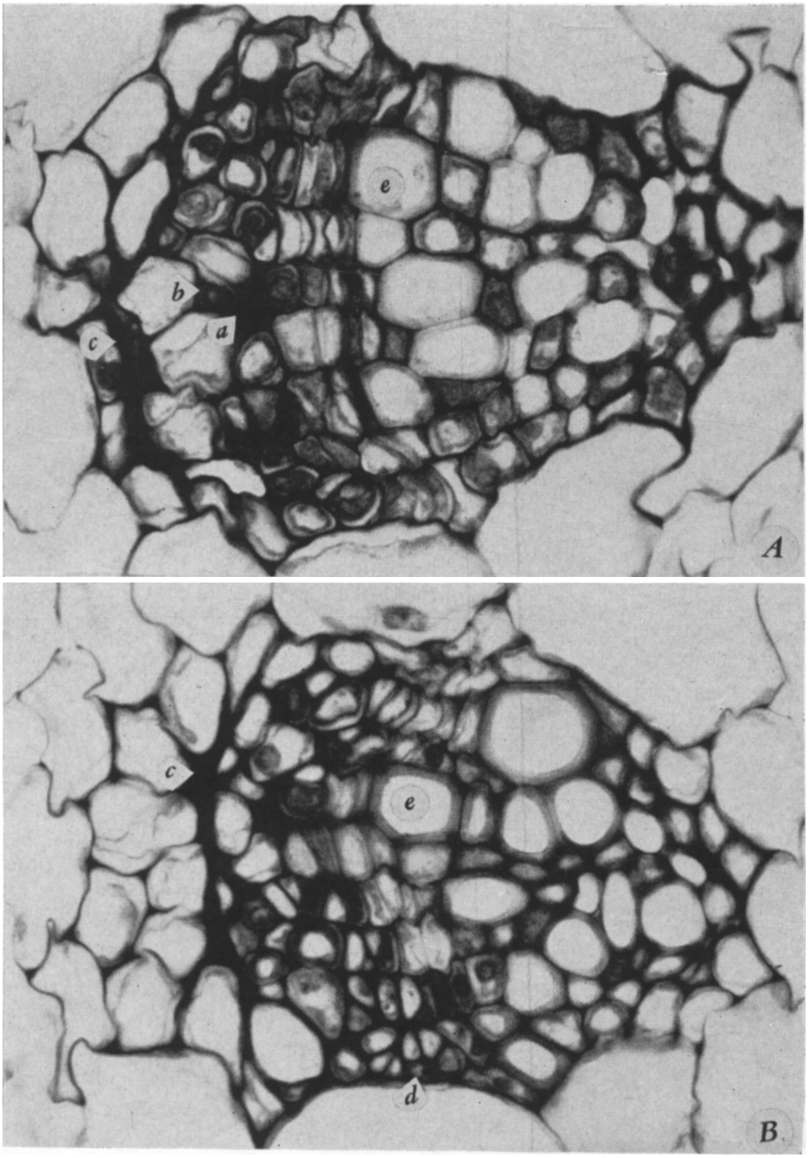

Fig. 5. Median vascular bundles from upper (*A*) and lower (*B*) parts of inoculated cotyledon from a plant collected three days after inoculation. *A*, Bundle with pronounced necrotic obliteration (*c*) and gum in sieve element (*a*); *B*, bundle with necrotic obliteration (*c*) and hyperplasia (*d*) at one side of phloem. Details: *a*, sieve element with gum; *b*, companion cell; *c*, necrotic obliteration; *d*, hyperplastic part of phloem; *e*, xylem element. (Both × 850.)

younger phloem, the vascular meristem, and the xylem, in that order. The small vascular bundles of the cotyledon (see fig. 6) showed no normal obliteration in accordance with their relatively late differentiation (cf. fig. 1).

The median cotyledonary bundle exhibited no hyperplasia in the collections made one and two days after the inoculation, but on the third day, hyperplasia was readily detected either in the lower part of the cotyledon or in both its lower and upper halves (cf. fig. 8). Figure 5 illustrates the situation when hyperplasia was still absent in the upper part of the cotyledon (*A*) but present in the lower (*B*). The hyperplasia was developing in the younger phloem and was affecting the vascular meristem as well (*B*, at *d*).

In addition to the hyperplastic derangement, the median bundles of many inoculated cotyledons showed an extensive collapse of cells with an accumulation of gummous material,[5] that is, a necrotic obliteration, in the older part of the phloem (fig. 5,*A* and *B* at *c*). A comparison of figure 5 with the unaffected bundle in figure 4,*A* suggests that the necrotic obliteration in figure 5 has involved the sieve elements and their companion cells—and possibly some parenchyma cells—that were located on the outer margin of the active phloem. Cells nearing normal obliteration and younger cells were probably affected.

Some evidence of necrosis could be detected in the first and second collections as revealed by small, isolated areas with gummous accumulations in the older phloem of the median bundles (cf. fig. 8). In the upper parts of the cotyledons some of the gum was positionally related to feeding punctures. In figure 5, similar accumulations of gum are visible in the younger phloem to the right of the area of necrotic obliteration. In figure 5,*A*, this gummous accumulation was identified within the lumen of a sieve element (*a*).

In later collections the hyperplasia became well established in the median bundles. In some samples of the final collection, made twelve days after inoculation, the entire younger part of the phloem was hyperplastic and all of the vascular-meristem cells had differentiated into abnormal phloem (fig. 4,*B*). Some apparently normal sieve elements with companion cells were detectable here and there in the outer part of the hyperplastic tissue.

In some plants occasional small vascular bundles of inoculated cotyledons began to develop symptoms at the same time as the main bundles. Later collections showed a larger number of affected small bundles. Some of the small bundles contained conspicuously hypertrophied parenchyma cells (fig. 7,*B* and *C*). The hyperplastic tissue was not extensive (fig. 7,*B* and *D*), and developed mainly from the meristematic cells that were present between the phloem and the xylem (cf. figs. 6 and 7).

Necrosis was evident in some of the small veins (fig. 7,*C*). Sometimes such necrosis occurred along the path of the feeding puncture made by the insect (fig. 7,*A*) as well as at a short distance from it. Some necrotic areas did not appear to be related to feeding punctures.

Feeding punctures were found in the median veins also, but necrotic obliteration was not spatially limited to the feeding-puncture areas. In fact,

[5] "Gummous material" and "gum" are used here in a wide sense to indicate the presence of deeply staining products of cell breakdown. The problematic nature of the definition and identification of gum is realized.

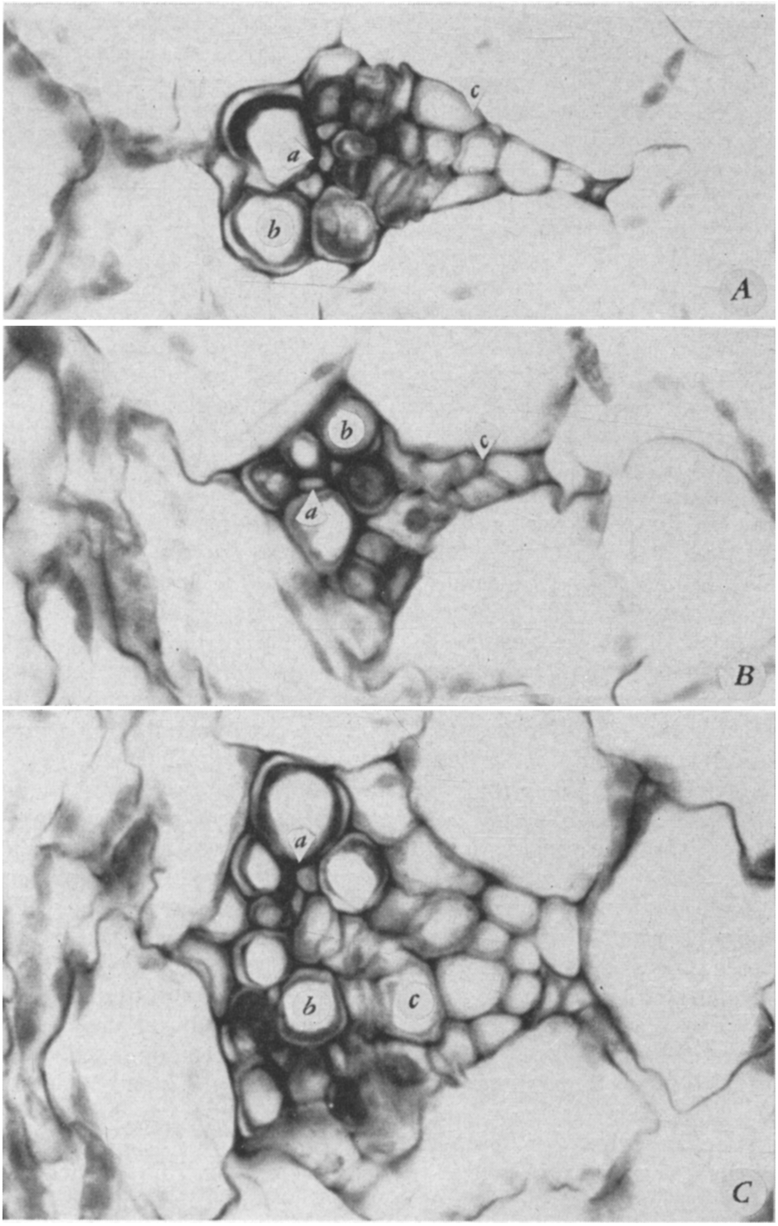

Fig. 6. Lateral cotyledonary vascular bundles free of histologic symptoms. *A*, Bundle from lower part of inoculated cotyledon from a plant collected one day after inoculation; *B* and *C*, bundles from upper part of inoculated cotyledon from a plant collected two days after inoculation. Details: *a*, sieve element; *b*, parenchyma cell; *c*, xylem. (All × 1,420.)

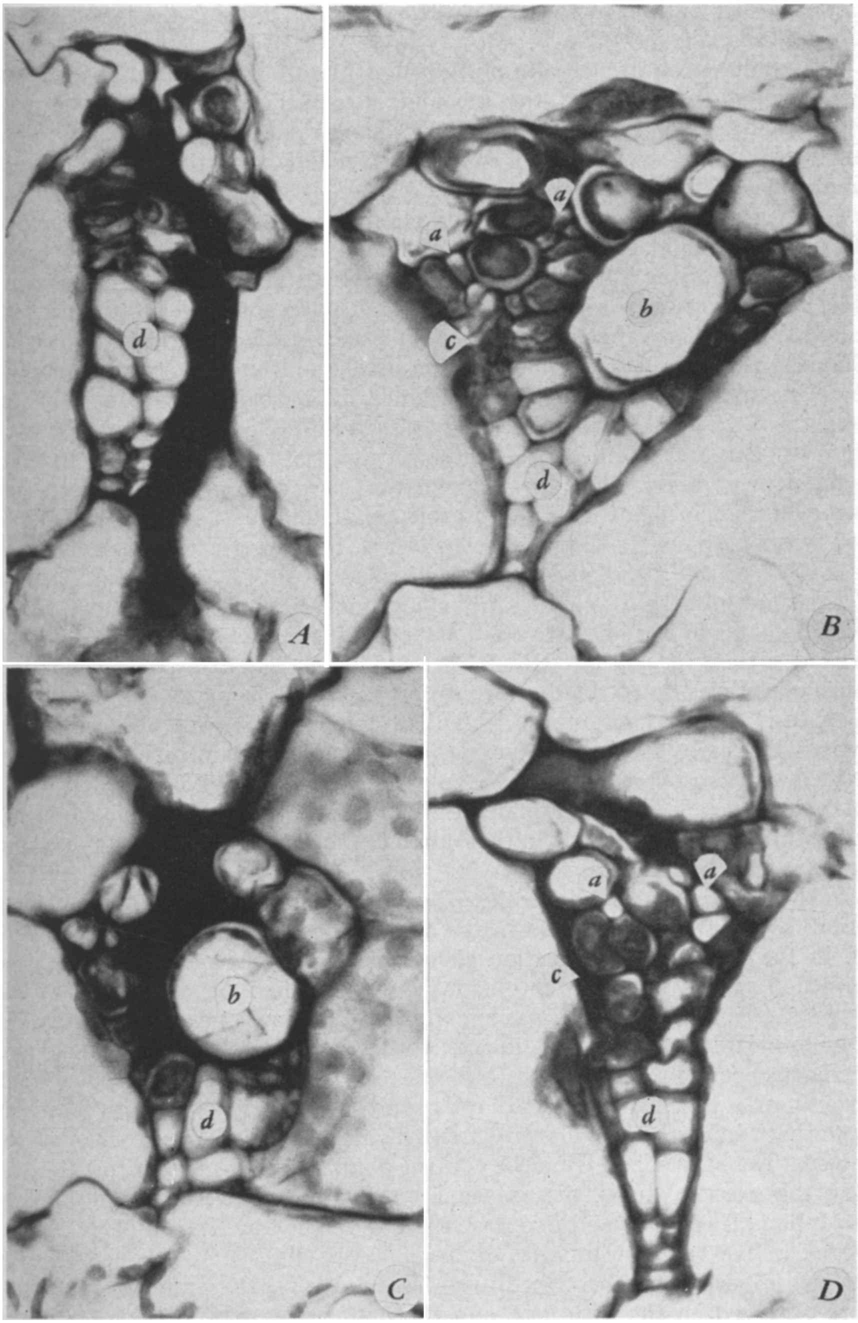

Fig. 7. Lateral cotyledonary vascular bundles from upper parts of inoculated cotyledons of plants collected three (*A*), four (*B*, *C*), and five (*D*) days after inoculation. *A*, Leafhopper feeding tract passing to right of xylem (*d*) and into phloem; *B* and *C*, hypertrophied parenchyma cells at *b*; *B* and *D*, mild hyperplasia at *c*. Details: *a*, sieve element; *d*, xylem. (All × 1,420.)

it was present in the lower part of the cotyledon to which the insect had no direct access (fig. 5,*B*).

The prominence of necrotic obliteration in the early stages of symptom development distinguishes the inoculated cotyledon from the subsequent leaves that develop symptoms after the systemic spread of the virus. In such leaves, extensive necrosis and collapse of cells commonly occur after the breakdown of the hyperplastic phloem (Esau, 1935, 1941). Without further experimentation it is impossible to judge to what extent the feeding process of the insect might have injured the phloem. Because comparisons between the effect of feeding of viruliferous and nonviruliferous insects are not available, one cannot draw a positive conclusion that the necrotic obliteration resulted from the presence of virus in the tissue. The observation made earlier (Esau, 1935), that normal obliteration of old phloem elements occurred in those inoculated cotyledons that developed hyperplasia, is pertinent in this connection. It should also be recalled that, in the 1935 experiments, five leafhoppers were placed on each cotyledon in contrast to only one per cotyledon in the present study. Thus, less injury from insect feeding would be expected in the later experiment. It is not improbable, therefore, that necrotic obliteration was a response to the presence of virus, perhaps related to the high degree of susceptibility of the strain employed.

The timing in the development of symptoms is recorded in figure 8. In the collections made one and two days after the inoculation, two plants in each showed some gum in the median bundle. Three days after the inoculation, hyperplasia was detected in the inoculated cotyledons of all five plants, but in three of them it was limited to the lower halves. Necrotic obliteration accompanied the hyperplasia in most cotyledons, and was present in the three cotyledon halves that had no hyperplasia. Hyperplasia was detected in the first two foliage leaves of all plants in the third, fourth, and fifth collections; in the third leaf of one plant in the fourth collection; and in the fourth leaf of one plant in the fifth collection.

In the additional material collected seven and twelve days after the inoculation, five out of nine plants in the seventh-day collection, and one out of six in the twelfth-day collection showed no hyperplasia in the inoculated cotyledon. Some of these cotyledons, however, had necrotic obliteration. Hyperplasia, when present, was usually combined with necrotic obliteration. The noninoculated cotyledons in the twelfth-day collection showed no hyperplasia or necrotic obliteration, but one cotyledon out of six had a small amount of gum in two isolated cells, and two other cotyledons showed a somewhat denser than normal staining in the parenchyma cells of the phloem. The cotyledons from the control plants showed no abnormalities.

In the present study, the external symptoms were recorded in the first two foliage leaves on the third day, that is, on the day that hyperplasia appeared in the vascular bundles of these leaves (fig. 8). In the previous experiments with small beet seedlings (Esau, 1935), the external symptoms were observed on the fifth day, and the first evidence of phloem degeneration was detected two to three days earlier. Furthermore, in the 1935 experiments, the symptoms developed later in the inoculated cotyledon than in the younger leaves. The 1935 studies were carried out at Davis rather

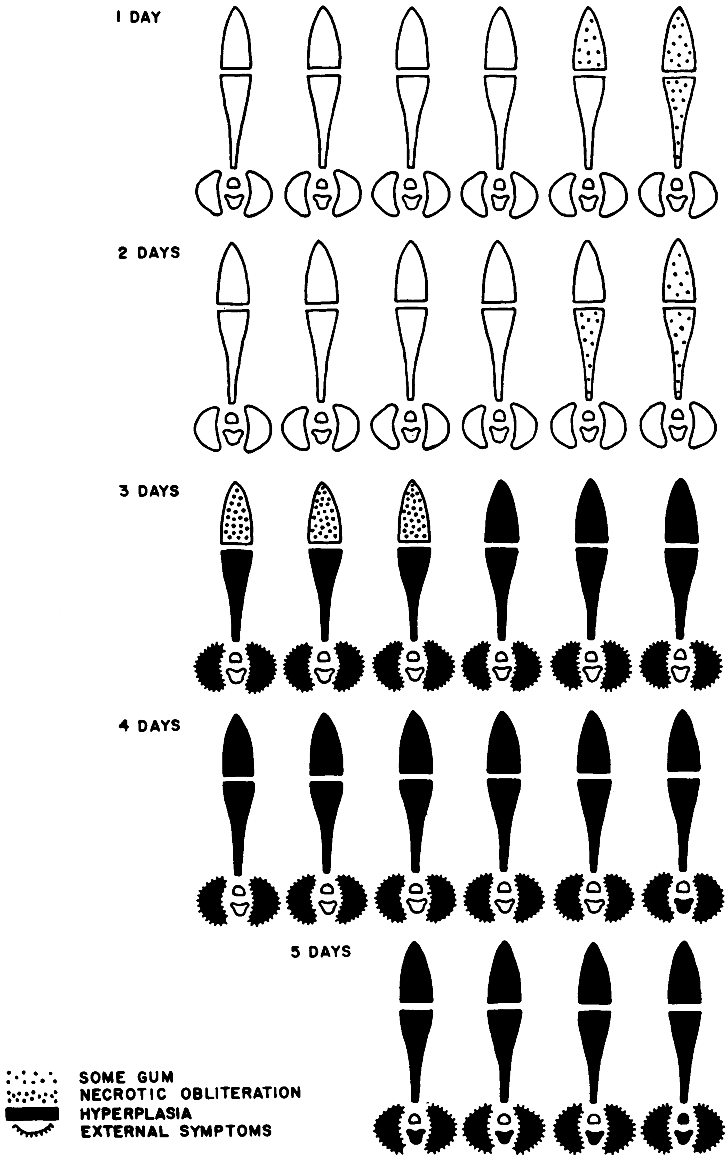

Fig. 8. Diagrammatic record of development of symptoms in seedlings inoculated through one cotyledon. The inoculated cotyledon, divided in two parts, is at the top in each drawing. The leafhopper was confined to upper half of cotyledon. The first pair of leaves, and leaves 3 and 4, appear below cotyledon in each drawing. The number of days at left refers to time interval between inoculation and sampling. Six plants were collected at the one- to four-day periods, four plants after five days.

than Riverside, the seed was from a different strain, and a larger number of insects was used for the inoculations. One or more of these deviations may have been responsible for the differences in timing of the internal and external symptoms in the two sets of experiments.

SUMMARY

Virus of curly top disease was introduced, through one cotyledon, into seedlings of a highly susceptible sugar beet strain. The inoculated cotyledon developed phloem hyperplasia—that is, abnormal multiplication of cells—characteristic of the curly top disease. On the third day after inoculation, the symptom was observed in the main vein and in some of the small veins. In many samples the main vascular bundle also showed pronounced necrotic obliteration in the older part of the phloem tissue, a symptom not previously described for curly top infection. The hyperplasia in the inoculated cotyledon and in the first two foliage leaves, and the external symptoms in the first two foliage leaves were all detected at the same time. Noninoculated cotyledons collected twelve days after inoculation of the experimental seedlings had developed no curly top symptoms.

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