Histological Changes Produced by Virus Yellows In Sugar Beet Leaves

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Greenhouse investigations, as well as extensive field observations of virus yellows in California, have been carried on by C. W. Bennett and A. S. Costa. They determined some of the common symptoms found in the field and a great variety of symptoms in the greenhouse. Histological studies of virus yellows were prompted by the unusual variety of symptoms appearing on infected beets in the greenhouse.

Information on tissues injured is an aid in identifying viruses.

Materials and Methods

The infected leaves for study of general yellowing in the field were obtained from a sugar beet field near Riverside, California. The greenhouse material was furnished by C. W. Bennett and A. S. Costa from sugar beets inoculated by aphids.

The fixed and sectioned material was stained with dilute hematoxylin, counterstained with safranin and sometimes in addition stained with light green in clove oil.

Fresh material was hand-sectioned in the usual way between pieces of corn pith and examined under the microscope.

Studies on Material from the Field

The most common field symptom of virus yellows is a general yellowing of older leaves. Veins are sometimes green at first, but eventually become entirely yellow. In addition these leaves become thickened and brittle with surfaces uneven, especially on the underside. Figure 1A shows a healthy leaf section and Figure 1B a yellowed section. Transverse sections of healthy, mature leaves show relatively even flat surfaces on both sides of the leaves. In the palisade area, the cells are long and loosely packed with rather large intercellular spaces as shown in Figure 1A. Similar sections of yellows-infected leaves show greater thickness with uneven, undulate surfaces. More striking effects are small, short, closely packed cells practically devoid of intercellular spaces. This condition prevails to some degree throughout the entire leaf. There was no sign of necrosis or degeneration visible in these yellows-infected leaves.

Studies of Symptoms of Material from the Greenhouse

Sugar beets inoculated with virus yellows in the greenhouse have shown a variety of symptoms, some of which are not common in field plants.

The following symptoms occur in the greenhouse: vein clearing, pinpoint cleared spots, and pimples with cleared spots which sometimes have necrotic areas in the center of some of the spots. Sometimes there is a form of etching on the upper side of veins which causes the veins to appear brown-

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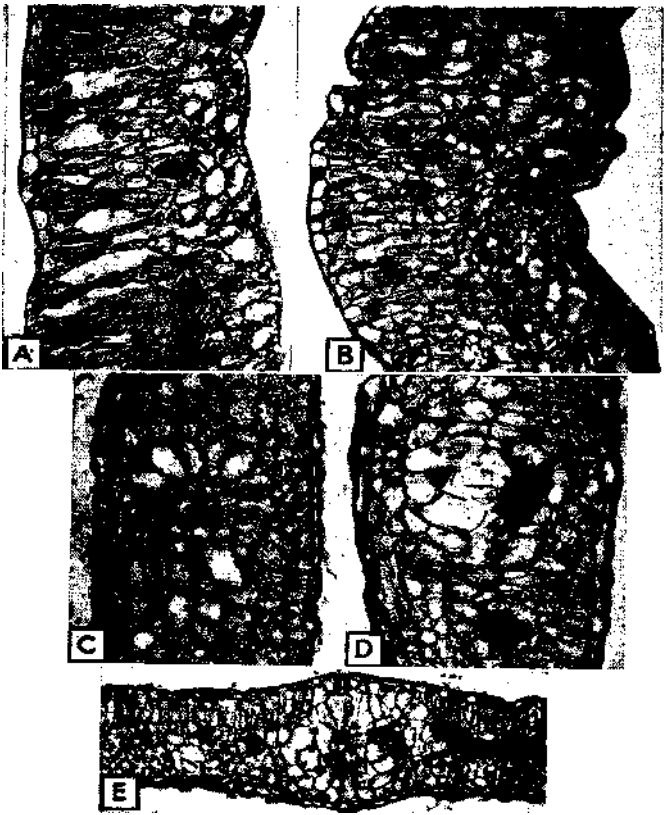

Figure 1.—Virus yellows symptoms in leaves of beets from field and greenhouse. A. Cross section of healthy leaf from field. B. Cross section of diseased leaf from field showing uneven surfaces, thickened condition and small closely packed cells devoid of intercellular spaces in palisade area. C. Cross section through small cleared vein showing necrosis in vein and enlarged cells almost devoid of chlorophyll. D. Pimple, showing greatly enlarged cells on lower side of necrotic vein. E. Pimple protruding on both surfaces of a beet leaf.

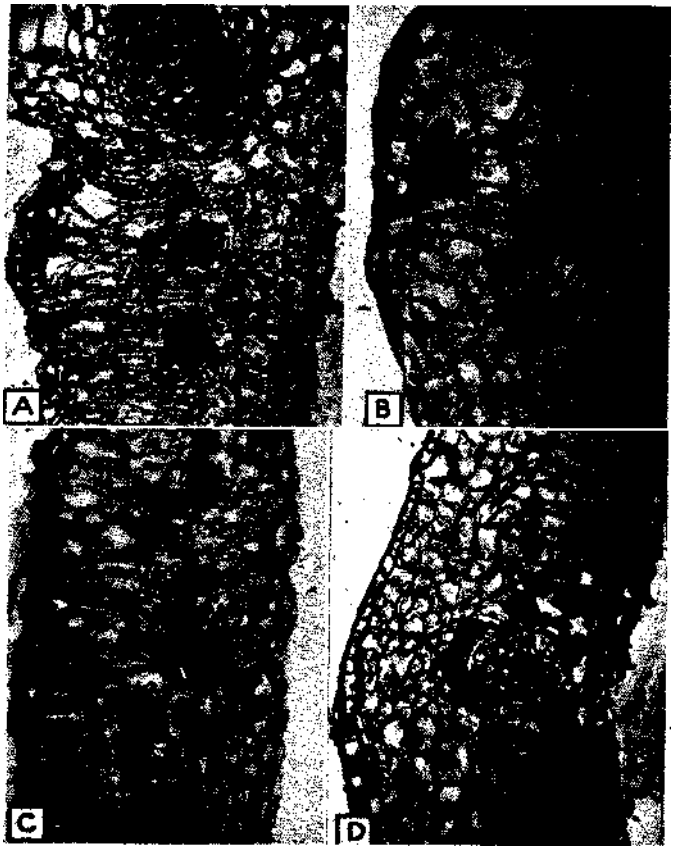

Figure 2.—Symptoms of virus yellows in sugar beet leaves. A. Necrosis of cells in large vein and beneath epidermis. B. Necrosis in large pimple involving parenchyma cells beneath epidermis. C. Necrotic spots in epidermis, and large cell inclusions of granular material (GM). D. Internal symptoms of "etch" showing necrotic cells following vein with necrosis in phloem in the side opposite necrotic parenchyma cells.

ish. There are also completely necrotic spots of various sizes in older leaves and irregular chlorotic spots known as flecking. The latter is a common symptom found in the greenhouse. Freehand sections of fresh leaves with vein clearing showed cells around the small veinlets to be considerably enlarged and almost devoid of chloroplasts. The chloroplasts present were not only fewer in number but smaller than in the tissues further away from the veins. In larger clear veins most of the cells of the lower protruding part of the veins had relatively few chloroplasts and little chlorophyll. Stained sections of fixed material showed necrosis in the bundles of the small cleared veins. In addition, the enlarged cells surrounding these were nearly devoid of chlorophyll, as shown in Figure 1C.

Pin point clear spots and pimples may result from inoculation of plants under greenhouse conditions. Pimples are produced, usually on the underside of the leaf (Figure 1D) , but some appear on both sides, as in Figure 1E. The pimples and cleared spots are associated with necrotic veinlets, and the hypertrophied parenchyma cells may be on one or both sides of the veinlet, thus producing pimples on one or both leaf surfaces.

Flecking seems to be due to destruction of chlorophyll in groups of parenchyma cells near a necrotic vein. This phenomenon is somewhat similar to "pinpoint" cleared dots, but without the excessive hypertrophy of parenchyma cells.

There are also necrotic lesions producing more conspicuous symptoms consisting of much larger necrotic areas on the leaf surface. These resemble the larger local leisons of some of the rubbed on inoculations of other kinds of virus diseases.

As seen in Figures 2A and 2B, there is necrosis in veins, phloem of large veins, and in parenchyma cells beneath the epidermis. In some cases this necrotic condition also affects the epidermis, as shown in Figure 2C. There was also a great increase in the number of large cell inclusions. These appeared to be composed of granular material (GM).

An unusual symptom designated as "dark etch" is characterized by dark brown lines over the veins on the upper surface of the older leaves. This is caused by necrosis of the epidermis and 2 or 3 layers of cells beneath. In some cases only the epidermis and a single layer of cells are involved, thus leaving 2 to 4 layers of unaffected cells between the necrotic area and the vascular bundle. The vascular bundle may show necrosis in the phloem of veins which show this etching effect, as illustrated in Figure 2D.

Summary and Conclusions

Virus yellows produces yellow, thickened and brittle leaves under field conditions. The greater thickness and increased brittleness of leaves appears to result from production of increased numbers of parenchyma cells which are so closely compacted that little intercellular space remains. No necrosis was observed in vascular bundles of leaves of field plants.

On greenhouse plants the disease produces pimples, vein clearing, etch, irregular chloretic and necrotic spots, yellow splotches, and some yellowing

of entire leaves. Vein clearing and pimples result from hypertrophy of cells immediately adjacent to veins. Since these hypertrophied cells contain little chlorophyll they produce translucent spots or lines and sometimes bulging of overlying tissue. Usually, at least a few necrotic cells were present in the phloem. Etching of the veins is caused by death of a certain number of superficial cells along the veins. Chlorotic or necrotic flecks of spots result usually from injury to epidermal and adjacent parenchyma cells in localized areas. Degree of injury determines the character of the spot. Spots superficially similar to flecks may be caused by groups of closely placed pimples. No cytological explanation of production of yellow spots and leaf yellowing, other than fading of chloroplasts in affected cells, was obtained.