Histology and Ultrastructure of Flax Crinkle

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ABSTRACT

Leaves of flax infected with the oat blue dwarf virus developed enlarged lateral veins on which enations were formed. Indentations of the leaf surface occurred opposite the enations on the adaxial side of the leaves. This excessive proliferation of tissues resulted from hyperplasia of phloem elements and hyperplasia and hypertrophy of cells that normally develop into fibers and chloroplast-containing parenchyma. Parenchyma cells associated with enations either had small and abnormal chloroplasts or were devoid of them. Changes in vascular bundles in stems were slight, and consisted only of some hyperplasia of phloem and hyperplasia and hypertrophy of fibers. Occasional phloem elements, fibers, and intercellular spaces were occluded with a dark-staining substance.

The virus was observed only in membrane-enclosed inclusions located along the inner walls of sieve elements, and was apparent as crystalline formations in some of these inclusions. Occasional membrane-bound inclusions were ruptured, and the virus was dispersed into the cell lumina. The virus particles were not inside, or associated with, cellular organelles. Phytopathology 61:1249-1252.

"Crinkle" of leaves is the most conspicuous symptom of infection of flax, *Linum usitatissimum* L., with the oat blue dwarf virus (OBDV). This symptom results from a swelling of lateral veins on the margins of leaves, small indentations along adaxial surfaces of veins, and enations on abaxial surfaces of the leaves (Fig. 1). The only leaves affected are those formed after exposure of the plants to viruliferous aster leafhoppers, *Macrosteles fascifrons* Stål. The first symptoms of infection in flax are visible ca. 10 days after the plants have been exposed to the leafhoppers. Other symptoms of this disease, as originally described by Frederiksen & Goth (2), include stunting, reduced boll development, and diminished seed set.

The diseases caused by the OBDV have not been studied histologically in any other dicotyledonous host, although the effects of the virus in oats, *Avena sativa*, have been thoroughly described (7). In oats, the virus was restricted principally to the phloem, but adjacent cells often underwent hyperplasia and hypertrophy. Vein swelling and enations along veins on the abaxial surfaces of leaves and leaf sheaths were prominent in oats. Hyperplasia of phloem elements and hyperplasia and hypertrophy of adjacent parenchyma were consistently associated with macroscopic symptoms. The virus was found in large crystalline formations in immature phloem elements. Mature sieve elements contained membrane-bound inclusions adhering to the walls, and virus particles scattered in the lumina of certain elements.

The objectives of this study were to examine by means of light and electron microscopy the histopathology and ultrastructure of OBDV-infected flax and the location and development of the virus in this host.

MATERIALS AND METHODS.—The flax cultivars, Marine 62 (C.I. 1661) and Redwood (C.I. 1130), were used in all experiments, and grown in a greenhouse maintained at ca. 21 C. The isolate of OBDV was originally transmitted from infected oats from the field to seedlings of Rodney oats in the greenhouse, where it was maintained by periodic transmission to seedling oats. Viruliferous aster leafhoppers were maintained continuously on OBDV-infected oats. Flax was inoculated by caging viruliferous leafhoppers for 1 week on seedling plants ca. 5-10 cm tall. The insects were then killed by spraying the plants with malathion in water, and the plants were kept in a greenhouse at ca. 21 C.

For histological studies that involved light microscopy, stems and fully expanded leaves of healthy plants and of OBDV-infected plants were sampled. Stem pieces ca. 1 cm long were excised from within 1-5 cm of the shoot apices; leaf pieces were 1 cm wide. All material was fixed in a formalin-acetic acid-alcohol fixative, dehydrated in a normal butanol series, and embedded in 56 C Tissuemat. Transverse and longitudinal sections were cut 8-10 μ thick and stained with Johansen's quadruple stain (4).

Fig. 1. Crinkle disease of flax. (Left) Oat blue dwarf virus-infected plant. Numerous enations along lateral veins on abaxial surfaces of leaves and indentations on the adaxial surfaces opposite the enations give the leaves the appearance called "crinkle". (Right) Healthy plant.

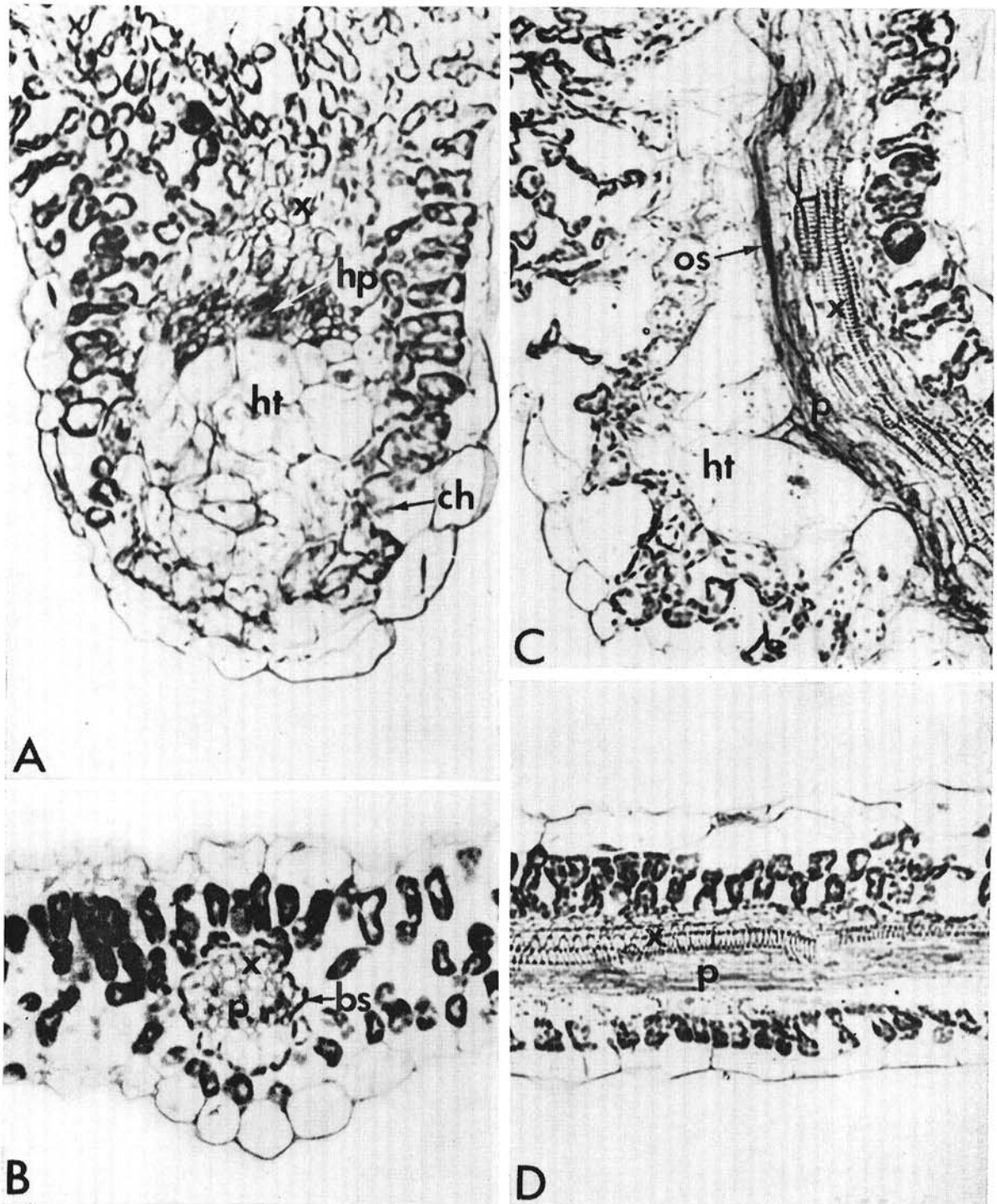

Fig. 2. Histology of **A, C)** crinkle-affected and **B, D)** healthy flax leaves. **A)** Transverse section of an enated lateral vein of an oat blue dwarf virus-infected leaf. Hyperplasia (hp) of phloem and hyperplasia and hypertrophy (ht) of phloem fibers and parenchyma abaxial to the phloem has occurred. Chloroplasts (ch) in hypertrophied parenchyma are absent or smaller and less intensely stained than are those in a healthy lateral vein. Xylem (x) apparently is not affected. **B)** Transverse section of a lateral vein of a leaf of healthy flax. Bundle sheath (bs) is absent in the diseased vein, but is present around the healthy vein (×240). **C)** Longitudinal section of enated lateral vein of oat blue dwarf virus-infected flax leaf. Darkly stained and disorganized areas in phloem (p) and obliterated sieve elements (os) were found consistently in enations. Hypertrophied cells (ht) developed abaxially to the phloem. Xylem (x) was not affected. **D)** Healthy lateral vein of flax (×240).

For electron microscopy of leaves, free-hand transverse strips, 1 mm wide, were taken from leaves near the shoot apex as well as from fully expanded leaves of healthy and diseased plants. Transverse stem sections, ca. 1 mm wide, were cut within 1 cm of the apices of diseased and healthy shoots and placed immediately in 6% glutaraldehyde-phosphate buffer (0.01 M, pH 7) for 12-18 hr at 4-5 C to fix the tissues. The material was then washed with buffer and postfixed with 2% unbuffered osmium tetroxide at 4-5 C for 6-18 hr. Tissues were dehydrated in graded acetone series, then embedded in Epon 812, and sectioned with glass knives. Sections were placed on 400-mesh uncoated grids and stained with uranyl acetate and lead citrate. Electron microscopy was done with a Philips EM-300 electron microscope.

RESULTS.—The first macroscopic symptoms of Crinkle occur in immature unfolded leaves of the shoot apex. Veins along the margins of the leaves become swollen, and the first enations appear. Although enations occasionally develop along the central vein, most of these are at the distal end of the leaf and are smaller than those on marginal veins. "Cupping" of the leaf usually occurs on the adaxial surface opposite the enations, and results in the leaf having an indented appearance. Several of these indentations and enations may occur in a row along each margin of an infected leaf and thereby give the leaf a "crinkled" appearance (Fig. 1). Enations do not occur elsewhere on infected plants, and except that the plants are stunted, there are no morphological changes apparent in the stems.

Light microscopy.—Transverse and longitudinal sections were obtained from fully expanded leaves of healthy plants, and from leaves with well-developed enations. Transverse sections of leaves with enations showed considerable hyperplasia of phloem elements, and hyperplasia and hypertrophy of phloem fibers and adjacent parenchyma (Fig. 2-A, B). Phloem fibers and chloroplast-containing parenchyma cells below the vascular bundle were greatly enlarged and distorted. Hypertrophied parenchyma cells were often devoid of chloroplasts and other subcellular constituents, or contained smaller and less deeply staining chloroplasts. Healthy vascular bundles were surrounded with a sheath of chloroplast-containing parenchyma cells, whereas diseased bundles in areas of enations were not delimited by such a sheath.

Short segments of vascular bundles were viewed in longitudinal sections (Fig. 2-C, D). Diseased phloem in enated areas was often disorganized and sometimes crushed. Some sieve elements appeared partially or totally occluded by a dark blue-black substance. Considerable hyperplasia of phloem and hypertrophy and hyperplasia of fibers and parenchyma cells below the vascular bundles also were noted in longitudinal sections.

Histological and anatomical changes in the vascular bundles of OBDV-infected stems were not so striking as those found in the large lateral veins of leaves. No enations or distortions other than a general stunting were seen in infected stems. Slight hyperplasia of phloem elements and some hyperplasia and occasional hypertrophy of phloem fibers were observed in sections of stems observed microscopically. Stem sections, like the leaf sections, had occasional sieve elements and phloem fiber cells partially or fully occluded by a dark blue-black substance. This dark-staining material was also observed in intercellular spaces.

Electron microscopy.—OBDV, which is a small (28-30 nm) polyhedron or sphere (1), was found only in sieve elements of lateral veins of leaves and in sieve elements of some vascular bundles in stems. Although the tissues were examined extensively, the virus particles were found only infrequently. The particles were detected most readily in the lateral veins of immature leaves located near the apex of the plant. Vein swelling was apparent along the margins of these leaves, and enations had begun to develop. In most of the cells that contained virus particles, the latter occurred in membrane-bound inclusions along the inner cell wall (Fig. 3-A). Occasional inclusions were found in which the membrane apparently had ruptured, so that the virus was released into the lumen of the cell (Fig. 3-B). OBDV inclusions were never found within any cellular organelles.

DISCUSSION.—OBDV in flax primarily affects phloem, phloem fibers, and parenchyma cells adjacent to the phloem bundles. Hyperplasia of phloem elements, together with hyperplasia and hypertrophy of fibers and adjacent parenchyma, are the most prominent anatomical changes due to virus infection. No virus particles were seen in fibers or parenchyma cells, although these tissues had developed hyperplasia and hypertrophy. The virus apparently multiplies only in phloem elements at some stage during their differentiation. The effects on adjacent tissues may be the result of a dysfunction of the phloem and the consequent impairment of translocation of growth substances.

The development of enations on the abaxial surface of large lateral veins on the leaf margins can be attributed to the hyperplasia of phloem elements and hyperplasia and hypertrophy of fibers and adjacent parenchyma. Excessive proliferation of these cells before the leaf has become fully expanded causes the protrusion of the abaxial leaf surface, and normal expansion of the remainder of the leaf causes the adaxial surface to become indented. A series of these abaxial enations and adaxial depressions along the large lateral veins of the leaf margins results in the crinkled appearance of the leaves. Westdal (6) pointed out that the development of enations was the most consistent and characteristic aspect of this disease in several host species.

Some of the symptoms of OBDV in flax are similar to those described by Severin & Houston (5) for flax infected with sugar beet curly top virus (SBCTV), which also is transmitted by a leafhopper. They reported that apical parts of SBCTV-infected plants were distorted, and that the leaves were wavy or convoluted. Leaf deformity of OBDV-infected flax apparently is more severe than is that reported for SBCTV infections. On the other hand, OBDV-infected flax remains

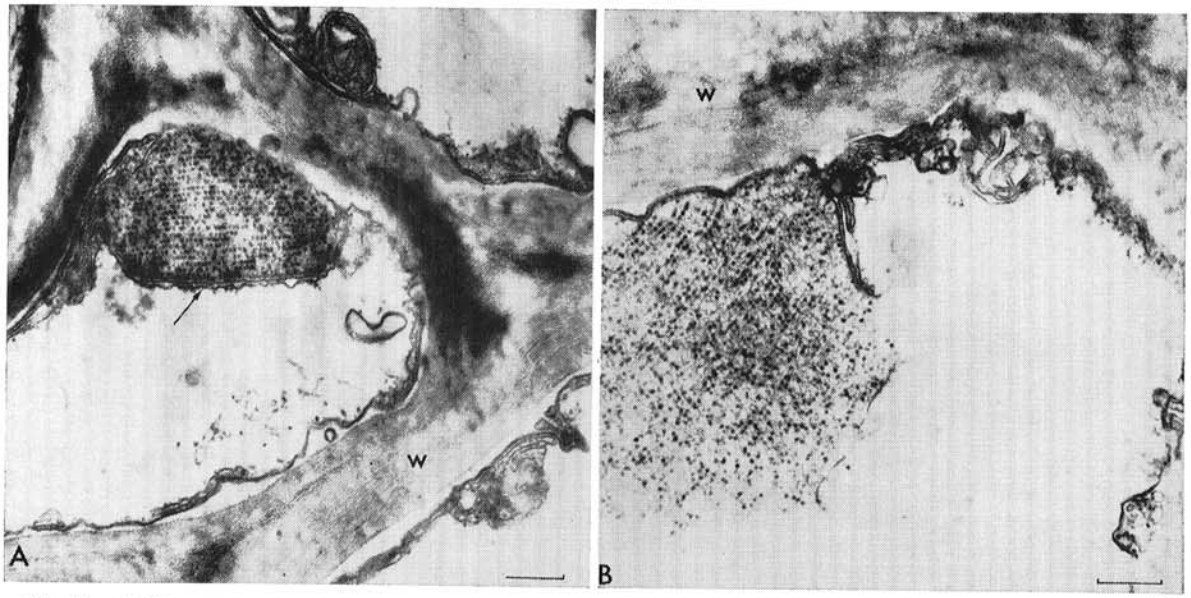

Fig. 3. Crinkle-affected flax leaves. **A)** Membrane-enclosed oat blue dwarf virus (OBDV) inclusion occurring along wall (w) of a sieve element. The virus was usually arranged into rows in these inclusions (Bar = 0.5 μ). **B)** OBDV inclusion along wall (w) of sieve element; inclusion membrane has ruptured and virus has been released into the lumen (Bar = 0.5 μ).

green until the plants mature, whereas SBCTV-infected flax gradually becomes yellow and dies.

Although hyperplasia of phloem elements and hyperplasia and hypertrophy of fibers and adjacent parenchyma are extensive in leaves of OBDV-infected flax, we did not observe necrosis such as that described in the phloem of leaves of SBCTV-infected flax (3). Moreover, the effects of OBDV on the vascular tissues of stems apparently were not so severe as those described for SBCTV infections of flax. The occasional occluded areas of hyperplastic phloem in OBDV-infected stems did not appear severe enough to cause gross disruption of phloem function. We did not find obliteration of sieve elements and the resulting formation of lucunae, as had been reported for SBCTV infections in flax stems.

That OBDV was found only infrequently in infected tissues suggests that the concentration of the virus in flax probably remains low. The membrane-bound structures which contained aggregated virus were found only in sieve elements, and were much like those reported to occur in OBDV-infected oats (7). It was apparent that the virus in these aggregates may be dispersed when the enclosing membrane of an inclusion ruptures and the virus is released into the lumen of the element.

There appears to be a special relationship between virus multiplication and symptoms in the lateral veins of leaves. If the multiplication of OBDV occurs in immature phloem of flax as was surmised in oats (7), then we might suspect that the greatest development of the virus in leaves of flax would be in the phloem of lateral veins of immature leaves. Morphogenically, these veins are the last to mature in flax leaves, and would have abundant immature phloem for virus multiplication.

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