Mycoplasmalike Bodies Associated with Witches'-Broom of Bleeding Heart

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ABSTRACT

Petiole and stem tissues of bleeding heart (*Dicentra spectabilis*) showing witches'-broom were examined with light and electron microscopes. Mycoplasmalike bodies were detected in the phloem elements, but were absent in the control healthy plants. These bodies were 50-600 nm in diam, and surrounded by unit membranes. Abnormal accumulation of callose in affected phloem cells, as compared with healthy ones, was observed by fluorescence microscopy. The constant association of these bodies with witches'-broom-affected plants suggested that they may be involved in the etiology of the disease.

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Additional key words: Yellows disease, ultrastructure, callose.

Bleeding heart (*Dicentra spectabilis* Lem.) is a herbaceous ornamental plant known for its winter hardiness and adaptability to various environmental conditions, and is common in home gardens in Canada and the northern states of the USA. The plants are easily propagated by root cuttings or clump divisions.

In October 1967, the incidence of a witches'-broom, hitherto unknown to the area, was recognized by a commercial grower who had been propagating bleeding hearts in an open field at Warburg, Alberta, Canada. Diseased samples (HD 67-725) submitted to the Crop Clinic Laboratory, Alberta Department of Agriculture, were forwarded to one of us (C. Hiruki) for identification of the causal agent. All the plants, including those received originally and some collected later from the same field, were maintained in the greenhouse. In attempts to determine the pathogen, mycoplasmalike bodies were found by electron microscopy in the phloem elements of affected bleeding heart plants. These pleomorphic bodies were morphologically similar to those observed in other plant species affected by yellows group diseases (1, 2, 6, 9, 10, 13, 14, 16, 17, 18).

MATERIALS AND METHODS.—Naturally affected and healthy bleeding heart plants were obtained from Warburg, Alberta. Both types were propagated by root cuttings in the greenhouse at 17 ± 2 C.

Phloem elements affected by the mycoplasmalike bodies were located by fluorescence microscopy (4, 7, 11). Young stems and petioles were sampled from affected plants and from comparable healthy plants. Thin free-hand sections were boiled for 3-4 min in water, then stained in 0.01% aniline blue in 1/15 M K_2HPO_4 (pH 8.0). Observations were made with a Leitz Orthoplan fluorescence microscope. A high pressure mercury vapor lamp (HBO 200 w) served as the illuminator. Two exciter filters (UGI, 2 mm), with maximum transmissibility at 366 nm, absorbed the visible spectrum. A barrier filter (K 430) was placed in the ocular tube of the microscope.

For electron microscopy, the excised parts (1-2 mm in length) were fixed in a 1:1 mixture of 3% glutaraldehyde and Formalin (pH 7.0) for 3 hr and postfixed in 2% phosphate-buffered (0.1 M, pH 7.0) osmium tetroxide for 2 hr. The tissues were then dehydrated through an ethanol series and embedded in Araldite. Thin-sections were cut with a glass knife on a Reichert OM2 ultramicrotome, and mounted on Formvar-coated copper grids. The thin-sections were post-stained in 2% (w/v) aqueous uranyl acetate, washed with water, then post-stained in lead citrate (15). Observations were made with a Philips 200 electron microscope at 60 kv.

RESULTS.—*Symptomatology*.—Healthy bleeding heart normally set only a few "eyes" per clump. The sprouts produced foliage ca. 1 to 2 ft high and normal-looking flowers (Fig. 1, left). The first symptoms in the affected bleeding heart was a slight yellowing or clearing of the veins of whole or parts of

Fig. 1-5. 1) Healthy (left) and diseased (right) bleeding heart plants (*Dicentra spectabilis*). Note stunting and witches'-broom symptoms with numerous fine shoots (× 1/3). **2)** A cross-section of a stem from a diseased bleeding heart showing the vascular bundle area. **3)** Note strong fluorescence in the irregularly shaped spots in the area of phloem degeneration (arrows). A few round fluorescent spots near the area of phloem degeneration indicate some of the cells which are filled with mycoplasmalike bodies. Fluorescence is due to callose deposition on the wall. C = collenchyma cells, X = xylem (× 300). **4, 5)** An enlarged portion of the tissue shown in Fig. 2. **4)** The cells with darker granulelike content are the ones filled with mycoplasmalike bodies. The obliterated phloem cells form the irregularly shaped thick-wall area (arrow). **5)** In ultraviolet light, this area fluoresces strongly, indicating accumulation of callose. Some of the phloem cells filled with mycoplasmalike bodies are recognizable by their fluorescence (× 750).


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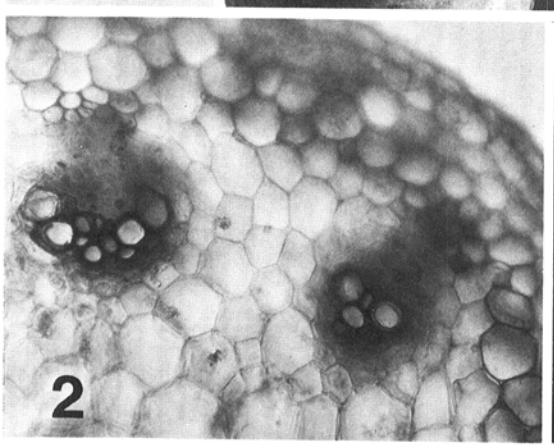
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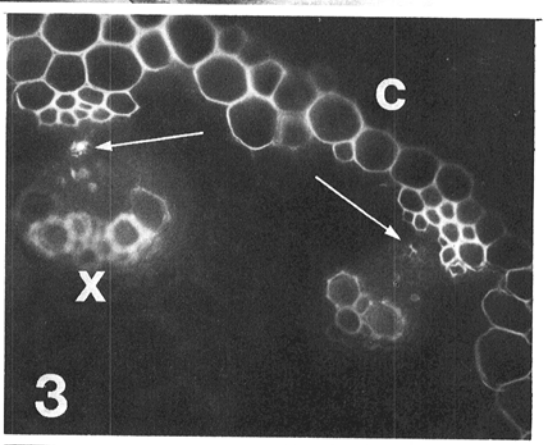
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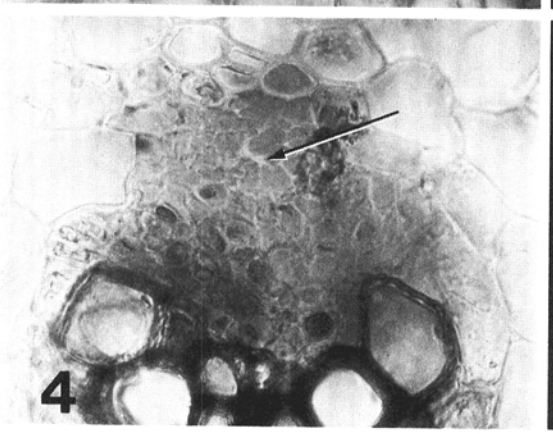
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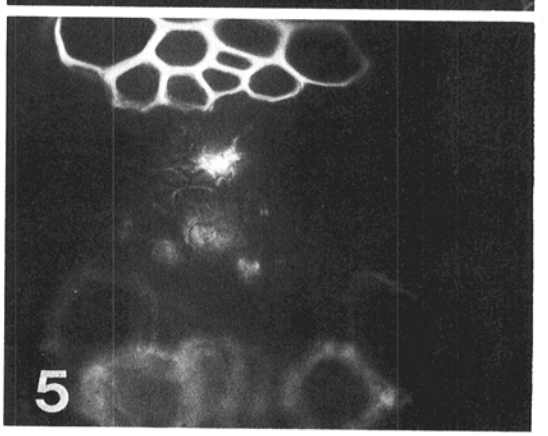
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young leaves. Such leaves often regained some normal green color at later stages of their growth. Affected plants were stunted and a single clump often produced 20 to 80 shoots which grew rather slowly and in turn produced numerous, slightly yellowish, upright, secondary shoots. The size and number of leaflets of a compound leaf were reduced, and the shape of such a leaf was irregular. The affected plants did not form floral organs (Fig. 1, right).

Mechanical transmission.—Mechanical transmission of a possible viral agent was attempted. Sap obtained in 1% K_2HPO_4, pH 8.5, at a 1:1 ratio (w/v) from affected plants was rubbed on selected healthy plants of several species after dusting them with 600-mesh Carborundum. Tested plant species included *Nicotiana tabacum* 'Bright Yellow', *N. glutinosa, N. rustica, N. debneyi, Chenopodium amaranticolor, C. quinoa, Gomphrena globosa, Datura stramonium, Petunia hybrida*, and *Phaseolus vulgaris* 'Red Kidney'. None of these plants showed symptoms.

Light microscopy.—Many examinations of leaf, stem, and root samples from affected plants failed to yield any evidence to indicate constant association of bacteria or fungi with witches'-broom of bleeding heart.

Since the symptoms suggested that a mycoplasmalike agent was involved, histopathological examinations were carried out. The examination of tissues stained with aniline blue fluorochrome permits specific detection of callose (4, 7, 11). Since callose occurs predominantly in phloem cells, and mycoplasmalike bodies are located mostly in phloem elements (1, 2, 3, 6, 9, 10, 13, 16, 17, 18), it was of interest to investigate whether this technique allows detection by the light microscope of possible abnormalities of cells affected by mycoplasmalike bodies, and, in particular, the effect of infection on callose accumulation in the phloem cells.

Twenty sections each from healthy and from affected bleeding hearts were stained with aniline blue and examined. Phloem elements of healthy samples were arranged regularly, showed a normal amount of callose deposition inside secondary walls of sieve tubes, and were free of necrotic cells. Affected samples, however, were somewhat irregular in arrangement of phloem cell elements and showed necrotic degeneration of certain phloem cells which revealed strong fluorescence (Fig. 2, 3). A few mature phloem cells were filled with granular materials which later were shown by electron microscopy to be morphologically identical to mycoplasmalike bodies (Fig. 4, 5). Accumulation of callose, judged by the intensity of ultraviolet fluorescence, was abnormally higher in affected phloem elements (19 of 20 samples examined) than in healthy ones. Thus, detection of the cells affected by mycoplasmalike bodies was made easier by this method.

Electron microscopy.—No virus particles were found in the tissues of affected bleeding heart plants. When the vascular tissues were examined, however, there were mycoplasmalike bodies in the phloem elements of affected plants (Fig. 6, 7), but not in those of healthy plants. Mycoplasmalike bodies were restricted to phloem elements, mainly in mature sieve tubes devoid of recognizable cellular organelles, and only a small number of phloem cells observed in cross-section contained the pleomorphic bodies. Distribution of the bodies in a given sieve tube was often irregular when tangential sections were examined (Fig. 10). Phloem cells adjacent to the affected ones were often free of bodies (Fig. 6).

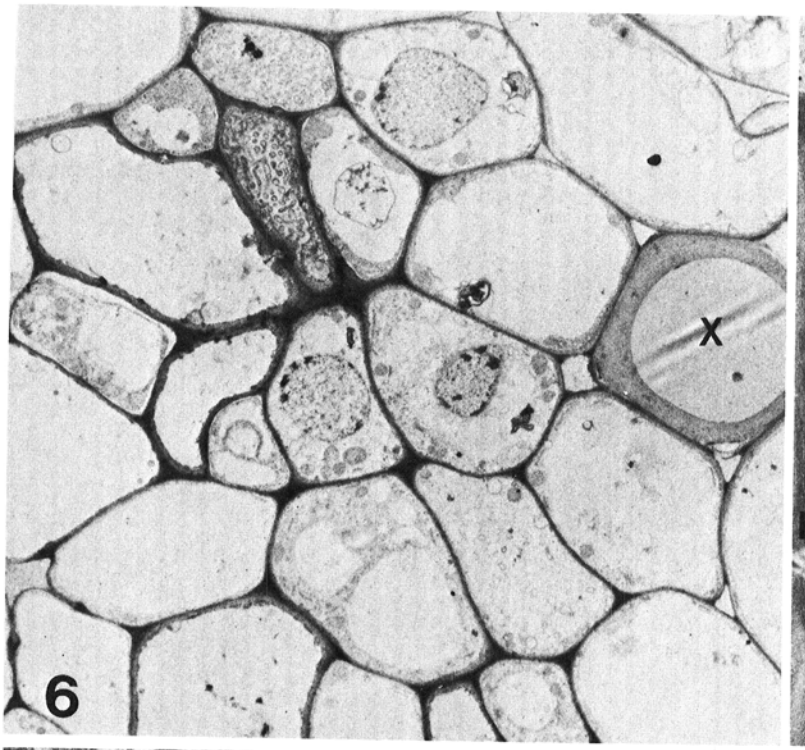
There was a wide variety of forms and sizes of bodies. Each body was bound by a poorly defined membrane. Individual bodies were often intermixed with strands of slime (Fig. 7, 8). Those of one form were spherical to oblong, 50-600 nm in diam. The spherical forms were of two types. The first type was smaller in size (50-80 nm in diam) and was electron-dense (Fig. 9, 11). The second type, larger in size, had an electron-transparent central area (Fig. 9, 11). A structure which suggested that the larger spheres underwent binary fission was often observed (Fig. 7, 8, 9, 12). A third type was represented by elongated bodies which often branched to various extents (Fig. 12). These were not uniform in width nor in length (50-200 nm × 500-1,100 nm), and often were constricted repeatedly.

The number of bodies within a section of a cell varied greatly (Fig. 11, 12). Occurrence of only a few mycoplasmalike bodies per cell was not uncommon. On the other hand, some phloem elements were filled with numerous bodies (Fig. 7).

In some mature sieve tubes, mycoplasmalike bodies were found in close association with the sieve pores (Fig. 10), suggesting that cell-to-cell movement of bodies could take place as pore sizes were apparently large enough to permit bodies to pass through.

DISCUSSION.—This study has shown that pleomorphic, mycoplasmalike bodies are closely associated with a witches'-broom of bleeding heart in

Fig. 6-12. 6) Electron micrograph of the vascular system in stem of diseased bleeding heart showing a phloem cell filled with mycoplasmalike bodies. Note also some of the phloem cells showing abnormally thickened cell wall. X = xylem cell (× 4,000). **7)** An enlarged phloem cell with mycoplasmalike bodies. Note irregularly thickened cell wall (arrows). Pd = plasmodesma (× 17,000). **8)** Pleomorphic or elongated bodies intermixed with strands of slime (arrow) (× 18,400). **9)** Spherical mycoplasmalike bodies of different sizes, mostly filled with electron semidense material (× 14,400). **10)** Mycoplasmalike bodies in the area of sieve-pores. The size of a pore is apparently large enough to permit the passage of the bodies (× 11,200). **11)** Spherical mycoplasmalike bodies along the cell wall of a phloem cell (× 43,200). **12)** A portion of a phloem cell containing characteristic mycoplasmalike bodies. Spherical, ovoid, elongated, and branched bodies are intermixed (× 52,000).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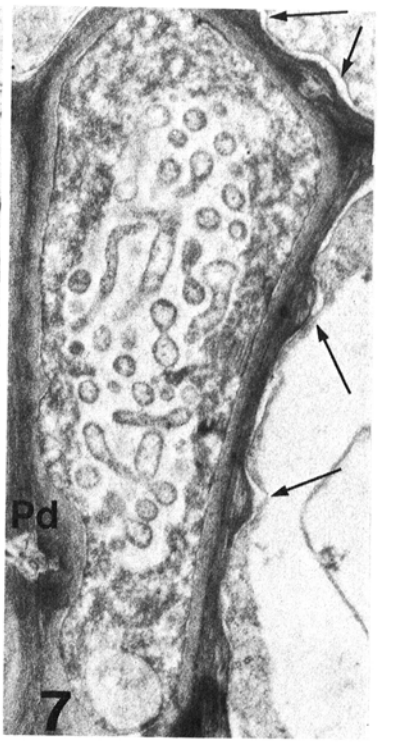
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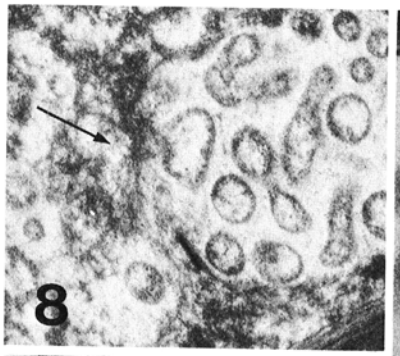
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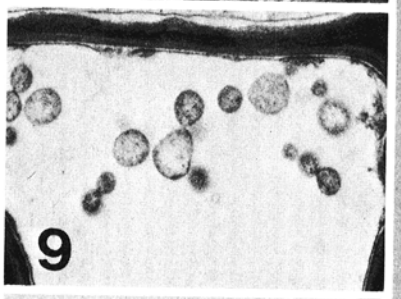
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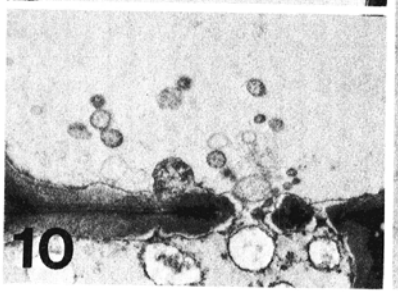
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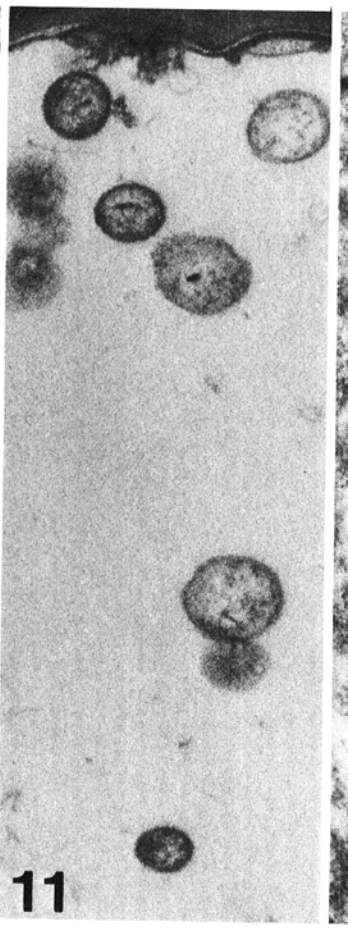
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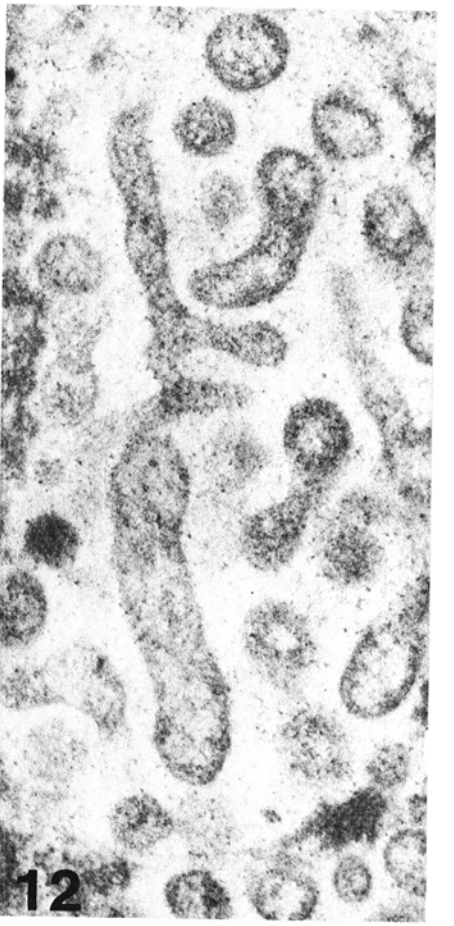
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Alberta. The presence of the bodies in diseased plants and their absence in healthy plants, although not direct proof, strongly suggests an etiological relationship of the bodies with the disease.

Mycoplasmalike bodies were observed in phloem cells of the diseased plants. Pleomorphism observed in this study was similar to that described previously by other workers (1, 2, 6, 8, 9, 10, 13, 14, 16, 17, 18), and was evidenced by a wide variety of sizes, shapes, structures, and electron densities. Bodies were surrounded by a poorly defined membrane but lacked cell walls.

On the basis of the characteristic growth habit, particularly the stunting and development of adventitous shoots, the name "witches'-broom" is proposed for the abnormality of bleeding heart described in this paper. No information is available on the geographical distribution of the disease other than that it is present in Western Alberta. The interrelationships of mycoplasmalike bodies observed in bleeding heart and those reported from other crops such as potato (1, 6, 16), clover, and barley (17) are not known at present, although certain yellows-type diseases including carrot yellows, potato witches'-broom (Hiruki, *unpublished data*), and clover proliferation (3) sporadically occur in Alberta.

A callose test, commonly known as the "Igel-Lange" test in certain countries in Western Europe (5, 12), has been used as a practical means for detection of potato leaf-roll virus infection which leads to abnormal accumulation of callose in sieve tubes. Our results indicated that the fluorescence microscopy technique employed in this study, when carefully applied, was useful in detection of abnormal accumulation of callose on the cell walls of plants affected by mycoplasmalike bodies. However, it should be borne in mind that callose is a normal constituent of sieve tubes (4, 5), and it thus may be of limited use in distinguishing affected cells from unaffected ones. Further studies will be required to determine whether this technique has practical value in diagnosing the yellows group diseases.

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