Mycoplasmalike Organisms in Sieve Tube Elements of Plants Infected with Blueberry Stunt and Cranberry False Blossom

Tseh An Chen

Associate Professor, Department of Entomology and Economic Zoology, Rutgers University, New Brunswick, New Jersey 08903.

The technical assistance of M. Tien is gratefully acknowledged, and the author wishes to thank P. E. Marucci and A. W. Stretch for their cooperation.

Accepted for publication 23 September 1970.

ABSTRACT

Leaf tissues of plants infected with blueberry stunt and false blossom of cranberry were examined with the electron microscope. Mycoplasmalike organisms were found in the sieve tube elements, but were absent in the control healthy plants. These bodies ranged in diameter from 80-300 nanometers (nm) for cranberry false blossom and 160-700 nm for blueberry stunt. The constant association of these microorganisms with the diseased plants suggested the possible roles they play in the etiology of the two diseases. Phytopathology 61:233-236.

Blueberry stunt and cranberry false blossom are among the most serious diseases occurring in New Jersey and other wild or cultivated *Vaccinium* fields in the U.S.A. In 1931, Dobroscky (2) reported that 44 species of leafhoppers were collected from cranberry bogs, and only one of them, the blunt-nosed leafhopper, *Euscelis striatulus* Fallen, was able to transmit the disease. Tomlinson et al. (10) and later Hutchinson (5) proved that the sharp-nosed leafhopper, *Scaphytopius magdalensis* Prov., was the vector of blueberry stunt (5, 10). In both cases, the etiologic agents were suspected to be viruses because they were easily transmitted to healthy host plants through vectors, by grafting, and to certain indicator plants by dodder (5, 6). The symptoms, such as sterile and malformed floral parts, witches' brooms, stunting, and small mottled leaves have also led workers to categorize them in the yellows group of plant virus diseases. No viruslike particles, however, have been isolated or found in the diseased plant tissue. In studying the cytology of the vector, Dobroscky also concluded that no differences existed between healthy and viruliferous insects (2).

In an attempt to find the pathogens with electron microscopy, mycoplasmalike organisms were observed in the sieve-tube elements of infected blueberry and cranberry plants. These pleomorphic bodies were similar to those found in corn stunt (1, 4), aster yellows (3, 8), sugarcane whiteleaf (7), and other yellows-group diseases (11).

MATERIALS AND METHODS.—Dormant blueberry plants (*Vaccinium corymbosum* L. 'Jersey'), were collected from Pemberton, N.J., in November and December 1969 and February 1970. Samples of actively growing blueberry plants were taken in April, May, June, and July 1970. Naturally infected and healthy cranberry vines (*V. macrocarpon* Ait.) were collected from bogs in Chatsworth, N.J., in November 1969 before flooding and in June 1970 during the peak blossom period. Cuttings were made from the collected dormant plants in the greenhouse during the winter of 1969.

Samples of diseased and healthy plants were taken periodically from collected materials. Buds of dormant plants and young and old leaves of growing plants were used for electron microscopy. The excised parts, usually including mesophyll and vascular tissues, were fixed in 6% glutaraldehyde in cacodylate buffer (pH 7.0) for 2 hr and postfixed in 2% phosphate-buffered (0.1 M pH 7.0) osmium tetroxide for 2 hr. The tissues were then dehydrated through ethanol series and embedded in Epon 812. Thin sections were cut with a diamond knife and stained with uranyl acetate and lead citrate (9).

RESULTS AND DISCUSSIONS.—No virus particles were found in the leaf tissues of stunt-infected blueberries and false blossom-infected cranberries. When the vascular tissues were investigated, however, there were mycoplasmaiike organisms in the sieve tube elements of both diseased plants. In the case of blueberry, no mycoplasmalike bodies were observed in the dormant bud tissues. Occasionally, few scattered bodies were found in the phloem of young leaves from the cuttings or from plants collected in the early growing season (Fig. 1-A). In May and June, when leaf symptoms of stunt (cupping and mottling) became apparent, samples taken from these plants showed a definite increase in the number of mycoplasmalike bodies in the sieve tubes of the leaves (Fig. 1-B). Since cranberries are evergreen plants, mycoplasmalike organisms could be observed in infected plant leaves collected in late November 1969 as well as in the active growing season (Fig. 2-A, B). Generally, there were more bodies in each of the affected sieve tube elements in cranberry than in blueberry. In no case were mycoplasmalike bodies detected outside of sieve tube or observed in control plants.

The morphology of these microorganisms was similar to those mycoplasmalike bodies observed in other yellows-infected plants. Their shapes ranged from spherical to oval to irregular. Each body was surrounded by a single unit membrane, and its simple cytoplasm possessed ribosomelike particles and fibrillar networks of presumed nucleic acid (Fig. 2-A). Their sizes, however, were quite different. Mycoplasmalike bodies observed in the cranberries ranged from 80-300 nm in diam, while those that occurred in the blueberries were much larger, with a diam of 160-700 nm.

Mycoplasmalike organisms were not detected in blueberry dormant buds, but became abundant as symptoms

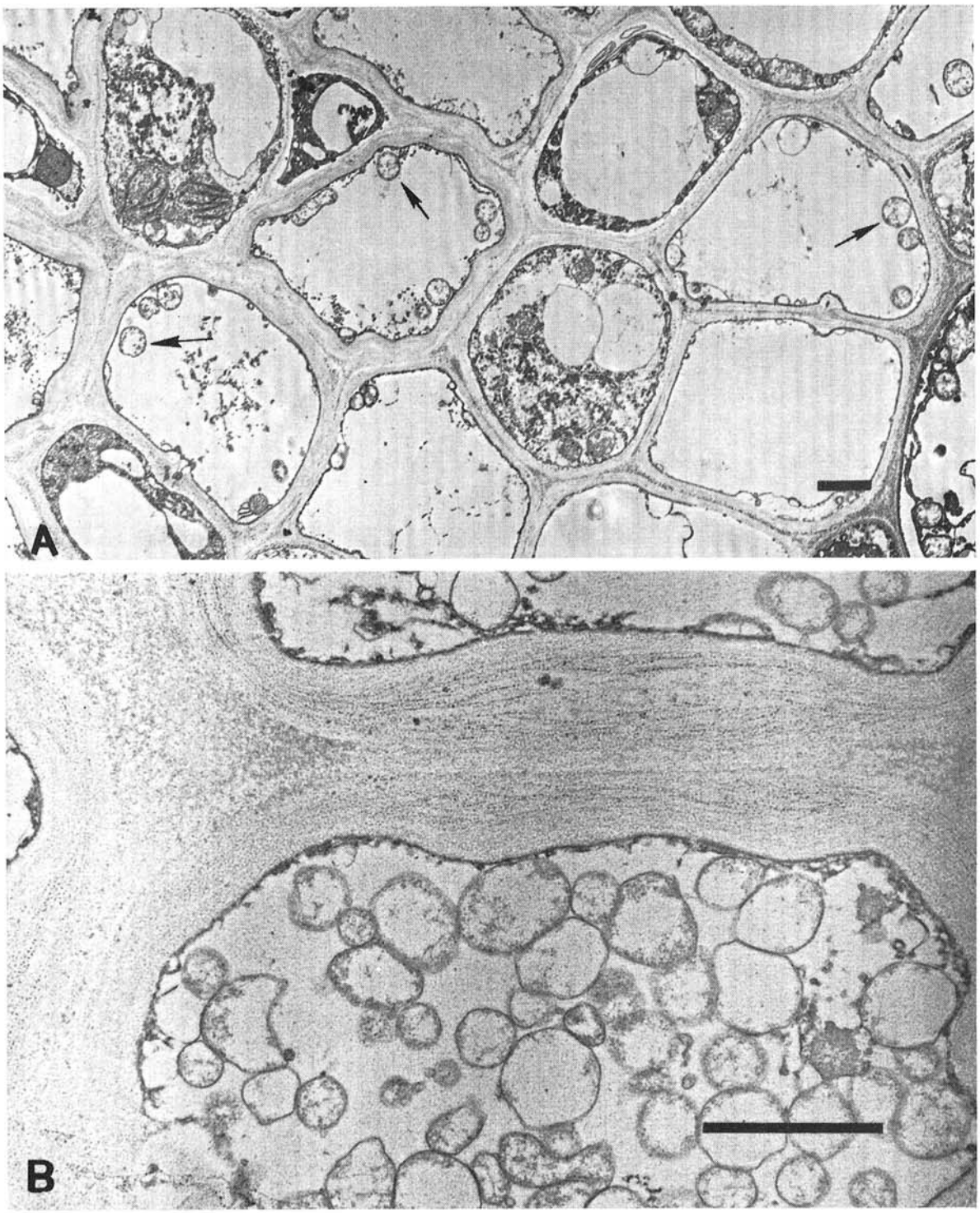

Fig. 1. Section of phloem tissue from leaves of stunt-infected blueberry plants showing **A)** scattered mycoplasmalike bodies (arrows) in the sieve tube elements of a young leaf; and **B)** a large number of mycoplasmalike bodies in a mature leaf. Bar equals 1 μ.

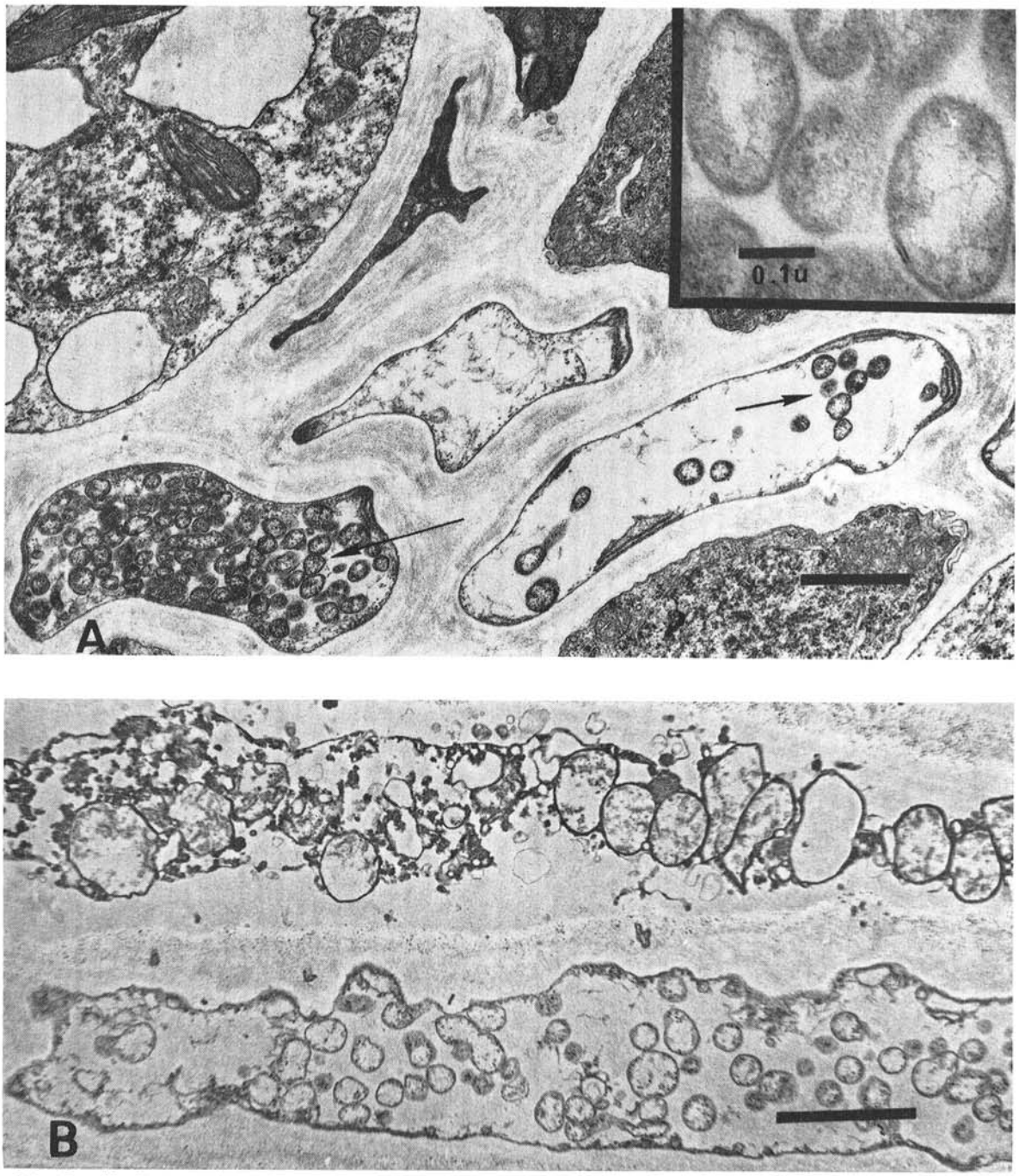

Fig. 2. **A)** Cross section of phloem tissue from leaf of false blossom-infected cranberry showing mycoplasmalike bodies (arrows) in the sieve tube elements. Inset: Close-up view showing strands of network inside membrane-bound bodies. **B)** Longitudinal section of a mature and a young developing sieve tube elements of diseased cranberry showing mycoplasmalike bodies in the mature sieve tube element. Bar equals 1 μ.

appeared. This suggests that these organisms may overwinter in the vascular tissue of the stems and roots. On the other hand, in cranberries, the flooding of the bog in the winter apparently protected the mycoplasmalike bodies in the infected leaves. Although these microorganisms have not been isolated in pure cultures, their constant association with the diseased plants indicates the possible roles they play in the etiology of the blueberry stunt and cranberry false blossom diseases.

LITERATURE CITED

1. CHEN, T. A., & R. R. GRANADOS. 1970. Plant-pathogenic mycoplasmalike organism: maintenance in vitro and transmission to Zea mays L. Science 167:1633-1639.

2. Dobroscky, I. D. 1931. Studies on cranberry false blossom disease and its insect vector. Contrib. Boyce Thompson Inst. 3:59-83.
3. Doi, Y., M. Teranaka, Y. Yora, & H. Asuyama. 1967. Mycoplasma or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom. Ann. Phytopathol. Soc. Japan 33:259-266.
4. Granados, R. R., K. Maramorosch, & E. Shikata. 1968. Mycoplasma: suspected etiologic agent of corn stunt. U.S. Nat. Acad. Sci. Proc. 60:841-844.
5. Hutchinson, M. T. 1955. An ecological study of the leafhopper vector of blueberry-stunt. J. Econ. Entomol. 48:1-8.
6. Kunkel, L. O. 1942. False blossom in periwinkles and its cure by heat. Science 95(2462):252.
7. Lin, S. C., S. C. Lee, & R. J. Chiu. 1970. Isolation and cultivation of, and inoculation with, a mycoplasma causing white leaf disease in sugarcane. Phytopathology 60:795-797.
8. Ploaie, P., & K. Maramorosch. 1969. Electron microscopic demonstration of particles resembling mycoplasma of Psittacosis-Lymphogranuloma-Trachoma group in plants infected with European yellows-type diseases. Phytopathology 59:536-544.
9. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-213.
10. Tomlinson, W. E. Jr., P. E. Marucci, & C. A. Doehlert. 1950. Leafhopper transmission of blueberry stunt disease. J. Econ. Entomol. 39:394-5.
11. Whitcomb, R. R., & R. E. Davis. 1970. Mycoplasma and phytarboviruses as plant pathogens presently transmitted by insects. Ann. Rev. Entomol. 15:405-464.