Investigation of Blueberry Stunt Disease in Arkansas

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ABSTRACT

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Electron microscopic examination of cultivated and wild blueberry plants exhibiting stunt symptoms in northwest Arkansas revealed that many contained mycoplasmalike organisms. Surveys in one commercial field indicated approximately 2.0% stunt-infected plants. Aniline blue staining of tissue and fluorescence microscopy were useful for distinguishing between stunt-infected and healthy plants.

The commercial acreage of highbush blueberries in Arkansas has grown from an initial 0.8 ha in 1969 to approximately 172 ha in 1980. During most of this period, the disease known as blueberry stunt—which is believed to be of mycoplasmal etiology (1,3)—was not observed in the area. In summer 1977, plants with symptoms suggestive of stunt were noted in two plantings. In late summer 1977, electron microscopic examination of tissue from a limited number of plants with stuntlike symptoms revealed a few mycoplasmalike bodies in one specimen (2).

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Cultivated blueberries (*Vaccinium corymbosum* L.) suspected of being infected with stunt in Arkansas displayed symptoms comparable to those reported for the disease in other blueberry growing areas in the United States (3,11). Typical symptoms included slight reduction in leaf size, marginal and interveinal chlorosis, cupping and puckering of leaves, and shortened internodes that gave plants a stunted and bushy appearance.

From 1978 through 1980, cultivated and wild blueberries in northwest Arkansas were examined for symptoms of stunt, and electron microscopic examinations were made to verify the association of a mycoplasmalike organism (MLO) with plants showing such symptoms. Total percentage of infection and differential susceptibility of cultivars in one commercial planting were determined, and fluorescence microscopic examination of tissue was investigated as an adjunct to disease diagnosis by symptomatology.

MATERIALS AND METHODS

Electron microscopy. Leaf vein tissues from both diseased and symptomless cultivated and wild plants were cut into 2- to 3-mm specimens and fixed in 4% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.2, for 2 hr. The tissue was postfixed in 1% osmium tetroxide in the same buffer for 2 hr, then stained overnight in 0.5% uranyl acetate. Specimens were then dehydrated in an ethanol series and embedded in Spurr's medium. Ultrathin sections were cut with diamond and glass knives on a Porter-Blum MT2-B ultramicrotome. Grids with thin sections were stained with uranyl acetate and lead citrate immediately after sectioning (8) and examined in a Siemens Elmiskop 1A electron microscope.

Field survey. An 8-yr-old commercial blueberry planting in northwest Arkansas was surveyed on 30 July 1979 for stunt disease. Each plant in the 4-ha field was examined for symptoms of stunt, and affected plants were tagged and mapped. The grower removed tagged plants in the fall of 1979. On 28 July 1980, another survey was made of the same field, and plants showing symptoms were recorded.

Wild blueberry plants (*V. pallidum* Ait.) in western Washington County, several miles from any cultivated blueberry plantings, were also surveyed for symptoms of stunt in late June 1979.

Fluorescence microscopy. Because fluorescence microscopy has been shown to detect excess callose in phloem of plants as a result of MLO infection (4),

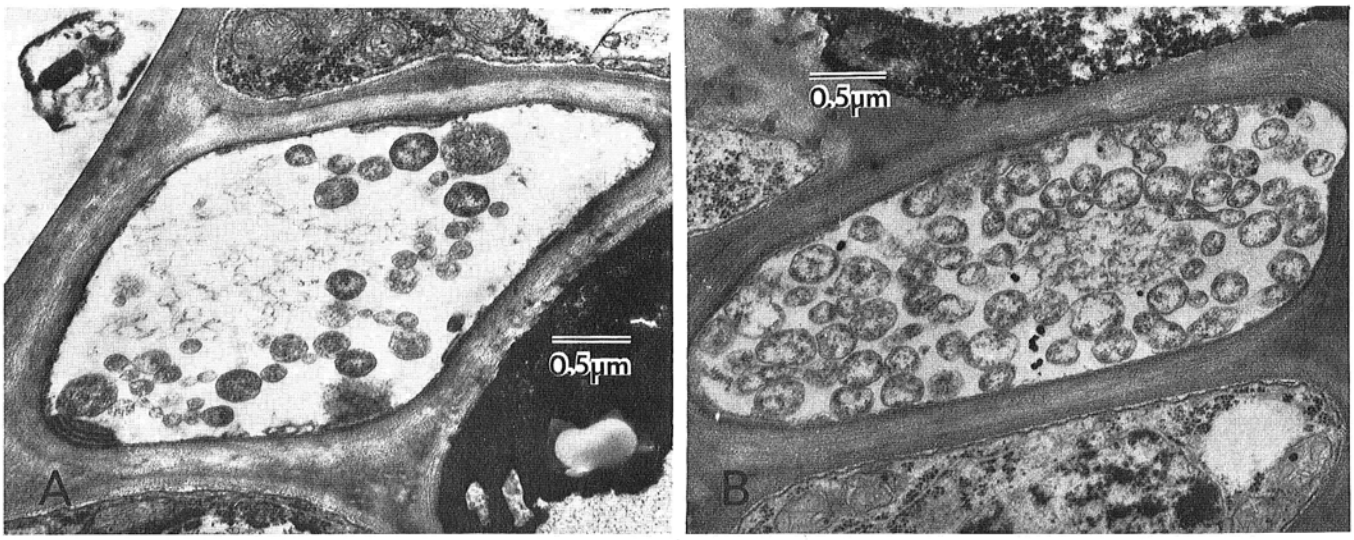

Fig. 1. Mycoplasmalike bodies in phloem cells of leaf vein tissue from stunt-diseased blueberry plants: **(A)** Cultivated blueberry (*Vaccinium corymbosum*). **(B)** Wild blueberry (*V. pallidum*).

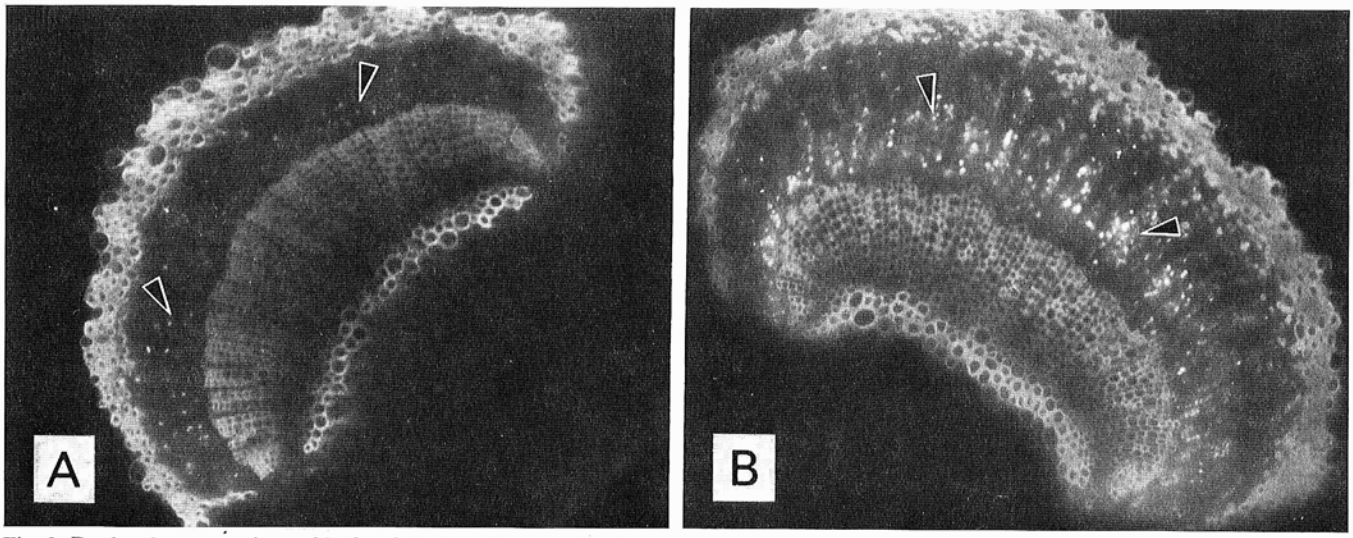

Fig. 2. Freehand cross sections of leaf petioles from healthy and diseased blueberry plants stained with aniline blue and viewed by fluorescence microscopy: **(A)** Petiole from healthy blueberry plant, showing only small amount of fluorescence caused by callose deposition in phloem (arrows). **(B)** Petiole from stunt-affected blueberry showing extensive fluorescence caused by callose deposition in phloem (arrows).

the technique was tested on cultivated and wild plants with stunt symptoms. Freehand sections 20–30 μm thick were cut from leaf petioles and fixed and stained with aniline blue according to Hiruki et al (4). Sections were examined with a Leitz Orthoplan microscope using a 200 W mercury arc lamp, exciter filters BG 38 and UG 1, and filter block H-2 (containing exciter filter BP390-490, beam splitter RKP510, and suppression filter LP515).

RESULTS

Electron microscopy. Electron microscopic examination of tissue collected from blueberry plants with stunt symptoms showed that 11 of 31 cultivated plants and 3 of 7 wild plants contained MLOs. Tissue from the cultivated plants (Fig. 1A) contained fewer MLOs than tissue from wild plants (Fig. 1B). MLOs were most readily seen in cultivated plants when tissue was collected in June and July. Tissue collected earlier than this period did not contain MLOs discernible in the electron microscopic sections, and that collected later often had degenerated phloem with no distinct MLOs.

Field survey. In the commercial blueberry planting that was surveyed in 1979, there were 230 plants showing symptoms of stunt. The pattern of the disease in the field was uneven, with slight differences observed in susceptibility between cultivars. Cultivars and observed infection percentages were Coville, 1.1%; Blueray, 3.3%; and Earliblue, 2.0%. After removal of infected plants in late 1979, the 1980 survey revealed an additional 120 plants with symptoms.

Stuntlike symptoms were observed in several wild plants examined in western Washington County. The symptoms were similar to those shown by Hutchinson et al (6) for stunt-infected *V. vacillans* Torr. in New Jersey.

Fluorescence microscopy. Fluorescence microscopy of aniline blue-stained leaf petiole tissue from stunt-infected plants showed considerably more fluorescence (Fig. 2B) than that exhibited by similar tissue from symptomless plants (Fig. 2A). Examination of tissue from both cultivated and wild plants during four time periods in 2 yr consistently revealed most fluorescence in tissue from plants showing symptoms.

DISCUSSION

The presence of MLOs in blueberry plants with symptoms of stunt in northwest Arkansas suggests that the disease is caused by a mycoplasma. From similarity in symptomatology, it appears that the disease is the same as blueberry stunt reported in other blueberry producing areas in the United States. The observation of MLOs in wild blueberries in the area suggests that the stunt disease is endemic in northwest Arkansas, with infected wild plants perhaps serving as a primary and continuing source of inoculum for infection of cultivated blueberries. This possibility is strengthened by confirmed presence in the area of the leafhopper *Scaphytopius magdalensis* (D. H. Johnson, *personal communication*), which is known to be a vector of the stunt organism in other parts of the United States (5,7).

The removal of diseased plants from the commercial field after the 1979 survey and the subsequent appearance of additional diseased plants in 1980 indicate that disease control by roguing must be a continuous process. The long-term effectiveness of roguing will be dependent upon recognition of plants showing only mild symptoms and determination of the distribution of a proven leafhopper vector in the area.

With the aniline blue technique, there appeared to be a difference in amounts of fluorescence shown by tissue from healthy and diseased plants. Verification of infection in plants, however, could perhaps be improved by use of fluorescence microscopy techniques that utilize reagents and stains specific for detecting MLOs in infected tissue (9,10).

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