A New Virus Disease of Yellow-Poplar

O. W. Barnett, Department of Plant Pathology and Physiology, and **V. B. Shelburne, Jun-Mei Yao,** and **F. H. Tainter,** Department of Forest Resources, Clemson University, Clemson, SC 29634-1003

ABSTRACT

Barnett, O. W., Shelburne, V. B., Yao, J.-M., and Tainter, F. H. 1996. A new virus disease of yellow-poplar. Plant Dis. 80:1317-1319.

Viruslike symptoms were observed on leaves of yellow-poplar (*Liriodendron tulipifera*) near the New River in Ashe County, North Carolina, in June 1987. Similar symptoms were seen in Oconee County, South Carolina, in 1988, and in Pickens County, South Carolina, in 1991. Many branches on the original tree in Ashe County had small leaves and shortened internodes. Leaves exhibited chlorotic veinbanding, stippling, and ring spots. Tissue from naturally infected trees was grafted to healthy yellow-poplar seedlings, and leaf symptoms similar to those on the naturally infected trees developed in the greenhouse. Sap inoculation from yellow-poplar seedlings to *Chenopodium quinoa* resulted in chlorotic local lesions and a faint general chlorosis. Leaf-dip preparations from both sources revealed short, straight rods; 74 particles from *C. quinoa* averaged 272 nm in length. This is the first viruslike disease described from yellow-poplar.

Yellow-poplar (*Liriodendron tulipifera* L.) is an attractive and commercially important tree of the eastern United States. Its range extends from southern New England across Michigan and south to the Gulf Coast into northern Florida. The center of its range is the southern Appalachians (North Carolina, Kentucky, and Tennessee), where it is abundant and reaches its largest size on mountain slopes, usually with a north or east aspect. Due to its wide geographic range, yellow-poplar grows in a variety of climatic and site conditions. Natural growth of yellow-poplar is best on moderately moist, well-drained, and loose-textured soils (2).

Diseases of the species are relatively few and are associated either with wounding or with weakening of the tree by some environmental stress. A viruslike disease of yellow-poplar was first noticed in 1987 near the New River in Ashe County, North Carolina. The disease was found in two separate sites in South Carolina in 1988 and 1991, respectively. Disease symptoms, graft transmission, and particle morphology are described more fully than in the initial report (1).

Current address of O. W. Barnett: Department of Plant Pathology, North Carolina State University, Raleigh 27695; E-mail: ow_barnett@ncsu.edu

Current address of Jun-Mei Yao: Department of Dermatology, New England Medical Center, Boston, MA 02111.

Accepted for publication 5 July 1996.

Publication no. D-1996-0924-05R

MATERIALS AND METHODS

Graft transmission via tissue implantation was performed by shaping a chip of wood from a small twig with symptomatic leaves into a thin double-wedge (4). Chips taken from tissue near the cambium were inserted into a slit made all the way through the stem of 2-year-old healthy yellow-poplar seedlings.

Sap inoculations were made by grinding young yellow-poplar leaves in a solution of 2% nicotine in water and rubbing on corundum-dusted leaves of healthy plants. Other sap inoculations were with a solution of 0.03 M 2-mercaptoethanol in 0.03 M sodium phosphate buffer, pH 8.0. Serological tests with antisera produced in the laboratory were by direct double antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA).

Partially purified preparations from young, infected yellow-poplar leaves were the source of virus for measurement in an electron microscope. Preparations were made by grinding leaves in 0.2 M potassium phosphate buffer, pH 7.0, containing 0.01% sodium thioglycolate. The extract was treated with an equal volume of chloroform:butanol, 1:1, given low-speed centrifugation, brought to a concentration of 0.34 M NaCl and 10% polyethylene glycol (PEG), and stirred 1 h prior to low-speed centrifugation. The pellet was resuspended in 0.05 M sodium citrate buffer, pH 7.0, and the PEG precipitation was repeated.

Viral preparations were applied to Formvar-coated grids and negatively stained with 2% (wt/vol) phosphotungstic acid, pH 7.0. The specimens were viewed with a Joel 100S transmission electron microscope at 60 kV. A carbon-coated grid, with 21,609 lines per mm, served as the calibration standard.

Ultrathin sections of yellow-poplar leaves from infected and healthy trees were prepared for transmission electron microscopy. Two-mm-square blocks of leaves were cut and fixed for 2 h in 2.5% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.2. Tissue was washed in buffer and post-fixed for 3 h at room temperature in 1% osmium tetraoxide. After washing with buffer, the squares were dehydrated by passing through a graded ethanol series. After two rinses in propylene oxide of 15 min each, the tissues were embedded in Poly/Bed 812. Ultrathin sections, cut with an LKB ultramicrotome, were stained with uranyl acetate and lead citrate and examined with a Joel 100S transmission electron microscope at 60 kV.

RESULTS

Disease description. A large yellow-poplar tree near the New River in Ashe County was noticed in June 1987 because many of the leaves were smaller than others (dwarfed) and new growth of many branches had reduced internodal lengths (stunted). Upon closer inspection, leaves had chlorotic ring spots and chlorotic stippling along veins (Fig. 1A). Only one tree along this fence had these symptoms in 1987 and 1988. When the site was revisited in June 1990 and 1994, several smaller trees (4 to 9 m tall) had the same symptoms. Root grafting could account for transmission to trees close to the original tree but not to those further away. Chlorotic stippling is a well-documented ozone injury on yellow-poplar (5), but the chlorotic ring spot is not and should preclude an ozone injury diagnosis.

In 1988, two native yellow-poplar trees in Oconee County, South Carolina, were found with similar symptoms. In May 1991, a number of large, native trees in a residential area in Pickens County, South Carolina, had these symptoms. No symptoms had been noted in the latter site from 1988 through 1990.

Graft transmission. Leaf symptoms similar to those on the naturally infected trees appeared 6 to 8 weeks after grafting. Older leaves often had green veinbanding symptoms (Fig. 1B). In some leaves, chlorotic sectors bound by veins occurred

(Fig. 1C). Stunting was not observed for any tree inspected in the greenhouse. In 1991, grafts from the yellow-poplar trees in Pickens County produced similar symptoms on seedlings.

Grafted trees have been maintained in the greenhouse for several years. New leaves can be forced most times of the year by removing old leaves and keeping the trees in light for 18-h days. Symptoms of chlorotic ring spots, chlorotic sectors bounded by veins, or chlorotic stippling along veins routinely occur in late June and July but can often be seen at other times of the year on newly emerged leaves.

Sap inoculation. Sap inoculations from newly emerged symptomatic yellow-poplar leaves were made to a range of plants. *Chenopodium amaranticolor* Coste & Reyn. showed local lesions on inoculated leaves. *C. quinoa* Willd. and *Belamcanda chinensis* (L.) DC. showed chlorotic local lesions and systemic chlorosis or spots. Small sugar pumpkin (*Cucurbita pepo* L.) had local lesions plus a systemic mosaic. *Datura stramonium* L. only showed a faint systemic mosaic. *Nicotiana sylvestris* Speg. & Comes exhibited a faint chlorosis, which was often difficult to distinguish. When sap from *D. stramonium* or *N. sylvestris* was inoculated to *C. quinoa*, typical local lesions developed. When *N. tabacum* L. 'Burley 21', *Zea mays* L., and *N. clevelandii* Gray were inoculated with sap from infected yellow-poplar leaves, no infection was observed. Symptoms could be perpetuated for only two or three successive transfers from *C. quinoa* to *C. quinoa*. Chlorotic symptoms often were so indistinct that they were barely noticeable unless they were compared to noninoculated plants.

Electron microscopy. No distinct viruslike particles were seen in thin sections of yellow-poplar leaf tissue, although

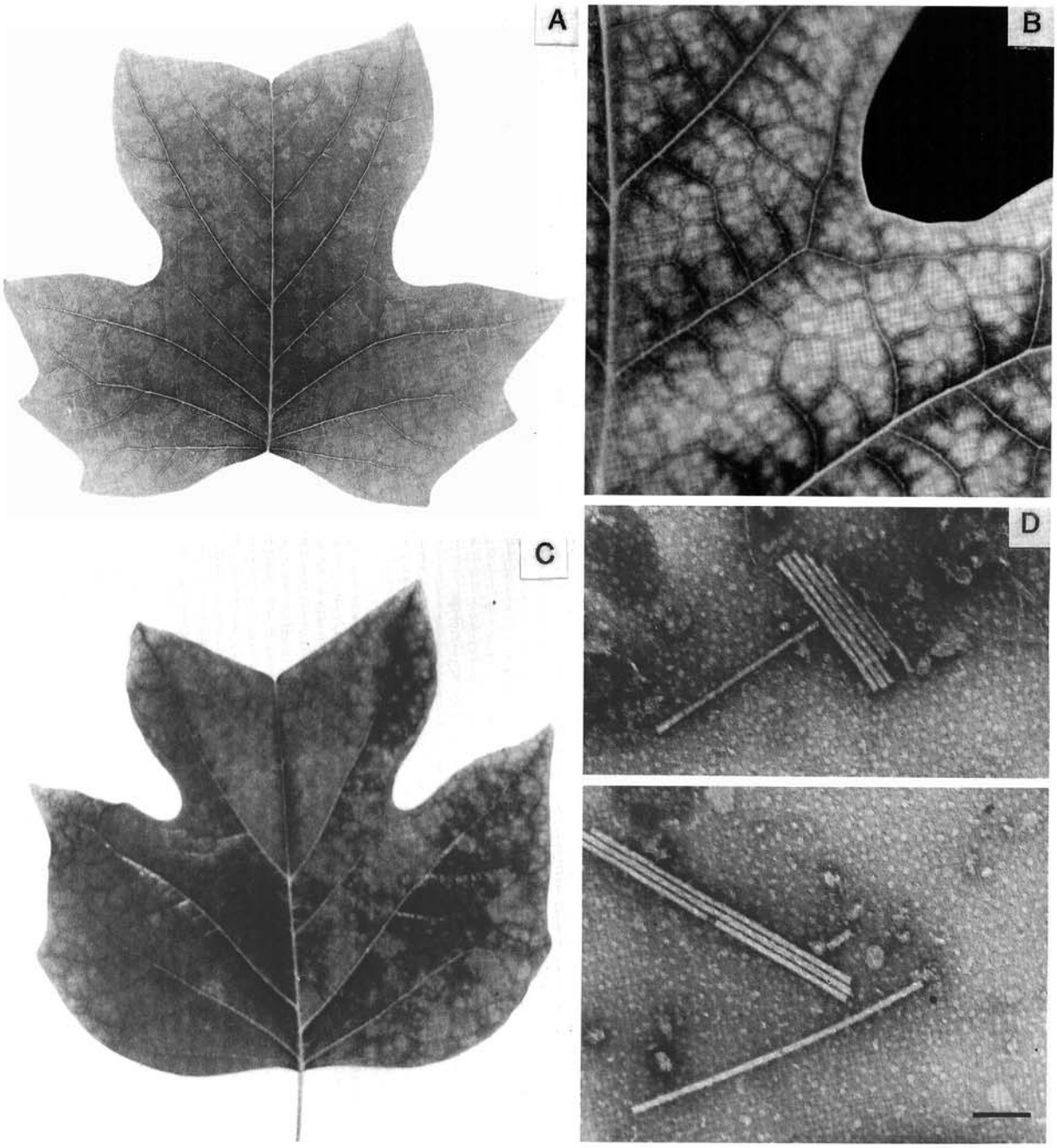

Fig. 1. Leaves of yellow-poplar with **(A)** chlorotic ring spots and chlorotic stippling, **(B)** green veinbanding, and **(C)** ring spots, chlorotic stippling, and chlorotic stippled sectors between veins. **(D)** Virus particles from partially purified preparations negatively stained with 2% phophotungstic acid. Bar = 100 nm.

electron-dense spherical bodies were initially thought to be virus particles (1).

Leaf-dip preparations in 2% sodium phosphotungstate were made from young yellow-poplar leaves and from leaves of *C. quinoa*. Stiff rods were seen in both preparations, but very few were found in leaf dips from yellow-poplar. Particles from *C. quinoa* were more numerous; after partial purification, 74 particles were measured, which averaged 272 nm in length (Fig. 1D).

Serology. Particles did not react with antisera made to tobacco mosaic, tomato mosaic, tobacco ring spot, tomato ring spot, tobacco etch, dogwood mosaic, Prunus necrotic ring spot, or prune dwarf virus. All homologous controls gave positive reactions.

DISCUSSION

Yellow-poplar is a commonly occurring tree in many parts of its range. Because of its rapid growth and ability to produce numerous sprouts and seedlings, it can usually overcome competition. Although this tree is intolerant of shade, its rapid growth allows the species to gain and hold its dominance in a stand. Large trees may be present in older home sites.

The original yellow-poplar that was infected prior to 1987 was still alive in 1994. Trees located in this fence-row did not have competition from all sides. The effect of this disease upon the persistence of yellow-poplar in a dense stand is not known, but if stunting is a common occurrence in natural settings, the disease could affect survival of this widely spread tree species.

Short range spread was documented at the Ashe County site. The source of inoculum for the Pickens County site is not known, but trees at this site were asymptomatic in 1988 and symptomatic in 1991. No systematic survey has been made, but symptoms were not observed in a few sites in Kentucky and other places in North and South Carolina.

Yellow-poplar is in the family *Magnoliaceae,* while poplar (*Populus*) is in *Salicaceae*. Poplar mosaic virus infects *Populus* and has a particle length of 675 nm (3), so the virus of yellow-poplar is not similar to poplar mosaic virus.

ACKNOWLEDGMENT

We thank Maria T. Zimmerman and Richard Baker for their assistance.

LITERATURE CITED

1. Barnett, O. W., Shelburne, V. B., Yao, J.-M., and Tainter, F. H. 1992. Virus disease of yellow-poplar. (Abstr.) Phytopathology 82:1174.
2. Beck, D. E. 1990. *Liriodendron tulipifera* L. yellow-poplar. Pages 406-416 in: Silvics of North America. Vol. 2, Hardwoods. R. M. Burnsand and B. H. Honkala, eds. U.S. Dep. Agric. For. Serv. Agric. Handb. 654.
3. Biddle, P. G., and Tinsley, T. W. 1971. Poplar mosaic virus. No. 75 in: Commonwealth Mycological Institute/Association of Applied Biologists Description of Plant Viruses.
4. Bos, L. 1967. Graft transmission of plant viruses. Pages 403-10 in: Methods in Virology, Vol. 1. K. Maramorosch and H. Koprowski, eds. Academic Press, New York.
5. Skelley, J. M., Davis, D. D., Merill, W., Cameron, E. A., Brown, H. D., Drummond, D. B., Dochinger, L. S., eds. 1987. Diagnosing injury to eastern forest trees: A manual for identifying damage caused by air pollution, pathogens, insects, and abiotic stresses. National Acid Precipitation Assessment Program, Forest Resources Program, Vegetation Survey Research Cooperative. For USDA Forest Service by Agricultural Information Services, College of Agriculture, Department of Plant Pathology, Pennsylvania State University, University Park.