Proc. Fla. State Hort. Soc. 109:24-25. 1996.

BAMBOO MOSAIC VIRUS DETECTED IN ORNAMENTAL BAMBOO SPECIES IN FLORIDA

M. S. ELLIOTT AND F. W. ZETTLER
Department of Plant Pathology
University of Florida
Gainesville, FL 32611

Additional index words. Dendrocalamus latiflorus, Bambusa dolichoclada, D. membranaceus, B. multiplex 'Fernleaf', potexvirus, direct tissue blot, ELISA, electron microscopy, light microscopy.

Abstract. **Leaves of *Bambusa dolichoclada* Hayata 'Stripe' and *Dendrocalamus latiflorus* Munro 'Mei Nung' with virus-like mosaic symptoms were collected from a nursery in south Florida. Epidermal strips stained with Azure A or calcomine orange/ Luxol brilliant green revealed cytoplasmic inclusions characteristic of the potexviruses. Of 129 flexuous rod-shaped particles observed in negatively stained leaf extracts, 88% were between 468 nm and 520 nm in length with a main maximum at 494 nm. Absorbance values (A_{405}) of infected *B. dolichoclada* and *D. latiflorus* 'Mei Nung' tissue analyzed by indirect ELISA using bamboo mosaic virus antiserum provided by N.-S. Lin (Institute of Botany Academia Sinica Nankang, Taipei, Taiwan ROC) ranged from 0.947 to 1.029 and 0.308 to 0.333, respectively. A_{405} values of healthy *Bambusa multiplex* (Lour.) Rausch. 'Fernleaf' Young and *Dendrocalamus membranaceus* Munro samples ranged from 0.406 to 0.516 and from 0.109 to 0.122, respectively. Strong positive reactions were observed on direct tissue blotted membranes. This is apparently the first report of bamboo mosaic virus in Florida and the second in the United States.**

In 1993, samples of two valuable bamboo species were collected to identify the agent responsible for causing the virus-like symptoms observed on the leaves. This study confirms that these samples were infected with bamboo mosaic virus.

There are 57 genera and more than 1200 species in the subfamily Bambusoideae. Bamboos are divided into two main groups based upon rhizome morphology. In general, the clumping or sympodial bamboos are tropical whereas the running or monopodial bamboos tend to grow in more temperate areas. Bamboos are valued for both their ornamental and edible qualities, and many species and varieties have been imported from Taiwan and elsewhere.

Bamboo mosaic virus belongs to the potexvirus group of viruses which are characterized by having flexuous rod-shaped particles ranging from 470 to 580 nm in length. Bamboo mosaic virus has no known natural vector and is mechanically transmitted through contaminated cutting tools used in vegetative propagation and harvesting of edible shoots.

Lin et al. (1977) apparently were the first to describe this virus infecting bamboo plants in Brazil. Lin et al. (1993) surveyed bamboos in Taiwan and detected bamboo mosaic virus in 11 species most of which belong to the genera *Bambusa* and *Dendrocalamus*. Whereas the first definitive report of bamboo mosaic virus-infected plants in the United States appeared in the literature only recently (Lin et al., 1995), bamboo collectors and enthusiasts apparently have observed virus-like symptoms on certain imported species for many years (Kim Higbie, Bamboosery, Miami, Florida; pers,. comm.). Based on the observation of flexuous rods using electron microscopy, bamboo mosaic virus was tentatively diagnosed in one sample of a *Bambusa* sp. with foliar mosaic symptoms submitted to the Division of Plant Industry in Gainesville in 1988.

Florida Agricultural Experiment Station Journal Series No. N-01327.

Materials and Methods

Plants of *Bambusa dolichoclada*, and *Dendrocalamus latiflorus* Young 'Mei Nung' with mosaic symptoms were collected from a nursery in Dade County, Florida. Abaxial epidermal strips were removed from leaves, stained with Azure A or calcomine orange/Luxol brilliant green and examined for virus-induced inclusions by light microscopy (Christie and Edwardson, 1977; Hiebert et al., 1984).

Leaf extracts were negatively stained with 2% uranyl acetate and examined with a Hitachi 600 electron microscope (Christie et al., 1987).

Gomphrena globosa L., *Cassia occidentalis* L., *Chenopodium quinoa* Willd. and *Dendrocalamus membranaceus* plants were dusted with 0.22 µm-mesh Carborundum and manually inoculated with extracts of infected *Dendrocalamus latiflorus* leaf tissue triturated in 0.02 M sodium phosphate buffer (pH 7.2). Inoculated seedlings were evaluated visually for symptom development. Extracts of local lesions which developed following inoculation were stained and examined with a Hitachi 600 electron microscope.

Infected *B. dolichoclada* and *D. latiflorus* 'Mei Nung' tissue and healthy *Bambusa multiplex* 'Fernleaf' and *Dendrocalamus membranaceus* tissue was analyzed by indirect enzyme linked immunosorbent assay (I-ELISA) using bamboo mosaic virus antiserum provided by N.-S. Lin (Institute of Botany Academia Sinica Nankang, Taipei, Taiwan ROC) (Yeh and Gonsalves, 1984). Leaf extracts were also blotted on nitrocellulose membranes and analyzed as described by Lin et al. (1993).

Results and Discussion

Two of 24 *G. globosa*, 1 of 7 *C. occidentalis*, 4 of 8 *C. quinoa* and 0 of 4 *D. membranaceus* plants manually inoculated with *Dendrocalamus latiflorus* leaf extracts developed local lesions. No systemic symptoms were observed. No particles were observed by electron microscopy in extracts of any of the local lesions presumably reflecting low virus titers in these local lesion hosts.

Cytoplasmic inclusions resembling banded bodies and paracrystaline bodies characteristic of the potexvirus group were observed when stained in Azure A or the calcomine orange/Luxol brilliant green combination (Christie and Edwardson, 1977.)

Flexuous rod-shaped particles characteristic of the potexvirus group were observed in negatively stained leaf extracts. Eighty eight percent of 129 particles measured were between 468 nm and 520 nm in length with a main maximum of 494 nm. This value falls within the range given for members of the potexvirus group and corresponds closely to the length of bamboo mosaic virus determined by Lin et al. (1977).

Absorbance values (A_{405}) of infected *B. dolichoclada* and *D. latiflorus* 'Mei Nung' tissue analyzed by indirect ELISA ranged from 0.947 to 1.029 (mean = 0.973) and 0.308 to 0.333 (mean = 0.317), respectively. A_{405} values of healthy *Bambusa multiplex*

'Fernleaf' and *Dendrocalamus membranaceus* samples ranged from 0.406 to 0.516 (mean = 0.456) and from 0.109 to 0.122 (mean = 0.112), respectively. While the healthy values were relatively high, the absorbance values of the infected plants were nevertheless at least two times the value of the healthy controls. Absorbance values from two to four times greater than the values of the healthy controls are commonly considered to be positive (Sutula et al., 1986).

Strong positive reactions were observed when sap from both infected bamboo species was blotted directly onto nitrocellulose membranes and incubated with bamboo mosaic virus antiserum. No reactions were observed between healthy bamboo sap and the bamboo mosaic virus antiserum, however.

Whereas bamboo mosaic virus has probably been present in the United States for many years, this is apparently the first definitive report of bamboo mosaic virus in Florida and only the second in the United States. The two infected bamboo species indexed in July 1993 had been imported from Taiwan and grown (apparently in quarantine) in California prior to being sold to collectors in Florida. Lin et al. (1995) also detected this virus in *Bambusa beecheyana* Munro growing at another location in California.

Inasmuch as this virus is mechanically transmitted, and bamboos are primarily propagated by division, the incidence of bamboo mosaic virus-infected plants is likely to increase. Due to the reproductive habits of bamboos, growers rarely have the opportunity to start virus free plants from seed. *Bambusa beecheyana*, a popular variety which is apparently widely infected with this virus, flowered and set seed several years ago, thereby providing the opportunity for growers to start healthy plants. Unfortunately "old generation", apparently virus-infected plants, are still being sold. Given the small numbers of growers and plants available in Florida, eradication is a possibility. However, growers are understandably reluctant to destroy their infected stock, and only a few have destroyed their virus-infected *B. dolichoclada* and *D. latiflorus* 'Mei Nung' plants. The best recourse to this situation is to follow good horticultural techniques such as isolating infected stock and using sterile cutting tools.

Bamboo mosaic virus symptoms range from very mild mosaic and striping on leaves to culm abortion. Thus, plant inspectors and quarantine officials may have difficulty identifying virus-infected plants. Careful inspection throughout the quarantine period is therefore necessary to prevent the importation of additional virus-infected bamboo species and varieties.

Literature Cited

Christie, R. G. and J. R. Edwardson. 1977. Light and electron microscopy of plant virus inclusions. Fla. Agr. Expt. Sta. Monogr. Ser. 9. 150 pp.

Christie, S. R., D. E. Purcifull, W. E. Crawford and N. A. Ahmed. 1987. Electron microscopy of negatively stained clarified viral concentrates obtained from small tissue samples with appendices on negative staining techniques. Fla. Agr. Exp. Sta. Tech. Bull. 872, 45 pp.

Hiebert, E., D. E. Purcifull and R. G. Christie. 1984. Purification and immunological analysis of plant viral inclusion bodies. Meth. Virol. 8:225-280.

Lin, N.-S., B.-Y. Lin, T.-Y. Yeh and Y.-H. Hsu. 1995. First report of bamboo mosaic virus and its associated satellite RNA on bamboo in the U.S. Plant Dis. 79:1249.

Lin, N.-S., Y.-J. Chai, T.-Y. Huang, T.-Y. Chang and Y.-H. Hsu. 1993. Incidence of bamboo mosaic potexvirus in Taiwan. Plant Dis. 77:448-450

Lin, M. T., E. W. Kitajima, F. P. Cupertino and C. L. Costa. 1977. Partial purification and some properties of bamboo mosaic virus. Phytopathology 67:1439-1443.

Sutula, C. L., J. M. Gillett, S. M. Morrissey and D. C. Ramsdell. 1986. Interpreting ELISA data and establishing the positive - negative threshold. Plant Dis. 70:722-726.

Yeh, S.-D and D. Gonsalves. 1984. Purification and immunological analyses of cylindrical inclusion protein induced by papaya ringspot virus and watermelon mosaic virus 1. Phytopathology 74:1273-1278.

Proc. Fla. State Hort. Soc. 109:25-28. 1996.

PRELIMINARY INVESTIGATIONS WITH FUMIGANT ALTERNATIVES TO METHYL BROMIDE IN FLORICULTURAL CROPS[1]

JAMES P. GILREATH
Gulf Coast Research & Education Center
University of Florida, IFAS
Bradenton, FL 34203

and

DANIEL W. WEST
Manatee County School District
Palmetto, FL 34221

Additional index words. Nutsedge, clover, sunflower, gladiolus, *Gladiolus* × *hortulanus*, *Helianthus annuus*, cut flower, weed control.

Abstract. **Dazomet, chloropicrin, 1,3-dichloropropene + chloropicrin, and metam-sodium were compared to methyl bromide combined with chloropicrin for weed control and crop response in preliminary field research with gladiolus (*Gladiolus* × *hortulanus* L.) and sunflower (*Helianthus annuus* L.) during 1995-96. Purple nutsedge (*Cyperus rotundus* L.) was controlled with methyl bromide, metam-sodium and dazomet. White clover (*Trifolium repens* L.) was controlled by chloropicrin and 1,3-dichloropropene + chloropicrin early in the winter; however, the time required to remove weeds manually from plots was greater with these two fumigants than with methyl bromide and was not different from the weeding time recorded**

[1]Florida Agricultural Experiment Station Journal Series No. N-01335. This research was partially supported by Manatee Fruit Co. Research results reported herein do not constitute a recommendation or endorsement by the authors, the University of Florida or the Manatee County School District.