HortScience 27(5):449-450. 1992.

# Shoot-tip Culture of Muscadine Grape to Eliminate Pierce's Disease Bacteria

**C.D. Robacker[1] and C.J. Chang[2]**
*Georgia Experiment Station, University of Georgia, Griffin, GA 30223*

*Additional index words.* *Vitis rotundifolia, Xylella fastidiosa,* propagation, tissue culture

***Abstract.*** **Pierce's disease, caused by the bacterium *Xylella fastidiosa* Wells et al., is widespread among muscadine grapes *(Vitus rotundifolia* Michx). To determine whether shoot-tip culture would be effective in eliminating *X. fastidiosa,* shoot tips of infected grape plants were cultured on Murashige and Skoog medium amended with 9 µM benzyladenine. Shoots and callus that developed tested negative for the presence of *X. fastidiosa.* Shoot-tip culture appears to be a promising method of obtaining muscadine grape plants free of Pierce's disease. Chemical name used: 6-benzylaminopurine (benzyladenine).**

Pierce's disease (PD) of grapevine, caused by the bacterium *Xylella fastidiosa* (Wells et al., 1987), blocks the xylem in nodes and petioles, inducing water stress in associated leaves and marginal leaf necrosis, leaf abscission, reduced vigor, and death. Pierce's disease is widespread among muscadine *(Vitis rotundifolia* Michx.) grapes, with sharpshooter leafhoppers acting as the vector. Although muscadines are generally more resistant to PD than are *Euvitis*, variability in resistance has been observed among cultivars (Mortensen et al., 1977). We observed typical symptoms of PD, including death, on susceptible cultivars grown in the greenhouse. *Xylella fastidiosa* was isolated from petioles of these plants several months before symptoms developed. Inadvertent spread of PD could occur when asymptomatic infected plants are propagated through the usual method of softwood cuttings. Grapes free of PD are needed for establishment of new vineyards in PD-free areas or for shipment of cuttings to PD-free areas. Muscadine grapes have been regenerated from shoot tips (Gray and Benton, 1991; Robacker and Lane, 1987). In this study, we micropropagated PD-infected muscadine plants through shoot-tip culture and demonstrated that the regenerated plants tested negative for presence of *X. fastidiosa.*

Fifteen greenhouse-grown plants of each of 'Triumph' and 'Noble' muscadine were inoculated with *X. fastidiosa* isolated from grapevines in Georgia (Chang et al., 1990). To obtain inoculum, the bacteria were grown in 20 ml of PD2 broth medium (Davis et al 1980) for 7 to 10 days at 30C with continuous shaking. The suspension was centrifuged at 19,600× *g* for 20 min, and the pellet was resuspended in 5 ml of 0.1 M phosphate-buffered saline to achieve 3 to 4 × $10^9$ colony-forming units/milliliter. Three pulvini per plant were injected by needle with 0.1 ml inoculum. Six months after inoculation, the plants were tested for presence of *X. fastidiosa.* Half of the tested plants had PD symptoms. Petioles were collected along the length of the vines, from near the tip to the base of the plant. Each petiole was cut into very fine pieces in PD2 broth medium in a sterile petri dish. The suspension was then streaked onto the bacterial isolation media PD2 and CS20 (Chang and Walker, 1988). The plates were incubated at 30C for 1 month. Colony development was observed under a binocular microscope ( × 50). Two-thirds of the plants tested positive; the bacterium was detected in petioles collected from every part of the vine. *Xylella fastidiosa* was isolated from 60% of the plants that had no PD symptoms and from 90% of the plants with symptoms.

Received for publication 1 July 1991. Accepted for publication 13 Jan. 1992. A contribution of the Univ. of Georgia Agr. Expt. Sta., Georgia Station, Griffin. This research was supported by state and Hatch Act funds allocated to the Georgia Agr. Expt. Sta. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Dept. of Horticulture.
[2]Dept. of Plant Pathology.

Three plants each of 'Noble' and 'Triumph' that tested positive for *X. fastidiosa* were selected for shoot-tip culture. Shoot tips, 2 to 3 cm long, were removed from three to four branches of each plant and were disinfested for 20 min in 1% sodium hypochlorite. The shoot tips were trimmed to 4 to 5 mm in length and cut into three about equal segments that were coded from 1 to 3, counting back from the tip. Microscopic examination of the tip segment (segment 1) revealed the presence of the apical meristem and two or more leaf primordia. The fragmented shoot-apex procedure of Barlass and Skene (1978) was used to propagate the plants. Each segment was cultured in liquid medium consisting of Murashige and Skoog (1962) salts and vitamins, 30 g sucrose/liter and 9 µM benzyladenine. The cultures were incubated at 27 to 30C under 16 h of light provided by 110-W wide-spectrum fluorescent tubes (70 $\mu M \cdot m^{-2} \cdot s^{-1}$). After 1 month, the tissues were transferred to fresh medium as above, except it was solidified with 8 g agar/liter. Within the next month, shoot tip segments 1 and 2 of 'Triumph" and 1, 2, and 3 of 'Noble' proliferated into axillary bud cultures with callus and/or shoots. Twenty-four cultures survived, but only eight 'Noble' and four 'Triumph' cultures contained shoots or leaves.

Shoots (up to 4 cm long) and callus that formed were tested for the presence of *X. fastidiosa* on PD2 and CS20 media. None of the regenerated shoots or callus tested positive for *X. fastidiosa* on either medium. Apparently, the PD bacterium in the infected greenhouse plants had not migrated into the shoot tips before culture, despite the presence of tracheary elements in the shoot primordia, as revealed by histological studies. In a similar study, Burr et al. (1988) reported elimination of *Agrobacterium tumefaciens* from *Vitis vinifera* plants regenerated from shoot tips.

We conclude that shoot-tip culture is a promising method of obtaining muscadine grape stock plants free of PD. These stock plants would be excellent sources of cuttings for traditional propagation of grapevines in instances where plant material was being introduced into a PD-free area.

**Literature Cited**


Barlass, M. and K.G.M. Skene. 1978. In vitro propagation of grapevine *(Vitis vinifera* L.) from fragmented shoot apices. Vitis 17:335-340.

Burr. T.J.. B.H. Katz. A.L. Bishop. C.A. Meyers, and V.L. Mittak. 1988. Effect of shoot age and tip culture propagation of grapes on systemic infestations by *Agrobacterium tumefaciens* Biovar 3. Amer. J. Enol. Viticult. 39:67-70.

Chang, C.J., C.D. Robacker, and R.P. Lane. 1990. Further evidence for the isolation of *Xylella fastidiosa* on nutrient agar from grapevines showing Pierce's disease symptoms. Can. J. Plant Pathol. 12:405-408.

Chang, C.J. and J.T. Walker. 1988. Bacterial leaf scorch of northern red oak: Isolation, cultivation, and pathogenicity of a xylem-limited bacterium. Plant Dis. 72:730-733.

Davis, M.J., A.H. Purcell, and S.V. Thomson. 1980. Isolation medium for the Pierce's disease bacterium. Phytopathology 70:425-429.

Gray, D.J. and C.M. Benton. 1991. In vitro micropropagation and plant establishment of muscadine grape cultivars *(Vitis rotundifolia).* Plant Cell Tissue Organ Cult. 27:7-14.

Mortensen, J.A., L.H. Stover, and C.F. Balerdi. 1977. Sources of resistance to Pierce's disease in *Vitis.* J. Amer. Soc. Hort. Sci. 102:695-697.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Robacker, C.D. and R.P. Lane. 1987. Effect of media and gelling agent on micropropagation and somatic embryogenesis of muscadine grape. HortScience 22:1137. (Abstr.)

Wells, J.M., B.C. Raju, H.-Y. Hung, W.G. Weisburg, L. Mandelco-Paul, and D.J. Brenner. 1987. *Xylella fastidiosa* gen. nov., sp. nov: Gram-negative xylem-limited, fastidious plant bacteria related to *Xanthomonas* spp. Intl. J. Systematic Bacteriol. 36:136-143.