Proc. Fla. State Hort. Soc. 124:4–6. 2011.

Screening for Crown Gall Disease Resistance in Various Grapevine Cuttings Inoculated by *Agrobacterium vitis* C493 and C4612

Xia Xu[1], Jiang Lu*[1], Jeong Ho Roh[1, 2], Zhongbo Ren[1], and Hong Huang[1, 3]

[1]*Center for Viticulture and Small Fruit Research, Florida Agriculture and Mechanical University, 6505 Mahan Drive, Tallahassee, FL 32317*

[2]*Visiting Scientist, National Horticultural Research Institute, RDA, Suwon 440-706, Korea*

[3]*School of Library and Information Science , University of South Florida. Tampa, FL 33613*

Additional index words. bunch grapes, muscadine grapes, plant disease resistance

Crown gall of grapevines is an important disease worldwide. In Florida, crown gall is commonly found in grapes, apples, peaches, raspberries, and roses. This condition reduces vine vigor and causes substantial economic loss for growers and nurseries. In this experiment, we screened 6- to 7-year-old green (June) and dormant cuttings (December) of 29 commercially available muscadine grape cultivars (*Vitis rotundifolia*), five European bunch grapes (*V. vinifera*), and five Florida hybrid bunch grapes, and two French-American hybrids, and *Shuttlewuthii* sp. by inoculating with *Agrobacteria* C493 and C4612. Cuttings without inoculation and cuttings inoculated with sterilized distilled water were used as controls. Gall incident rate and gall weight were scored at inoculated sites and top sites of the cuttings 2 months after the inoculation. All the green cuttings from muscadine grape cultivars showed about 0.1 to 0.2 g callus/gall growth (abnormal overgrowth) in the inoculated sites for both C493 and C4612. Higher levels of abnormal overgrowth were found on cuttings inoculated with C4612. However, only inoculated cuttings from *V. vinifera* and Florida hybrid bunch grapes showed callus/gall growth ranging from about 0.2 to 0.3 g at inoculated sites with C493 or C4612. No callus/gall growth was noticed for controls. Scanning electron micrographic analysis of the growth tissue found the presence of bacteria in inoculated ones but not in controls for all cultivars screened. The results indicated that muscadine grape was not completely immunized to crown gall disease and pathogen C4612 was more virulent than C493.

Crown gall, an important soilborne bacterial disease, is caused by *Agrobacterium tumefaciens*, which infects more than 200 dicotyledonous plants such as apples, peaches, raspberries, and roses worldwide (DeCleene and DeLey, 1976). There are three recognized biotypes of *A. tumefaciens* based on pathogenicity: Biovar 1, 2, and 3. Biovar 1 strain has a very wide host range; biovar 2 is classified as a hairy root-forming organism commonly found in stone fruits, whereas biovar 3, also called *A. vitis*, is mainly confined to grapevines (Burr and Otten, 1999; Miller, 1988; Ophel and Kerr, 1990). Usually, *A. vitis* initiates infections at wounded areas of grapevine trunks, canes, and nodes, leading to abnormal overgrowth or galls, which may also develop at graft unions where buds have been removed from rootstocks (Burr et al., 1984, 1998; Sule and Burr, 1998). Galls are normally first observed in early summer and repeatedly appear later on injuries caused by winter freezing temperatures. Cultivars of *Vitis vinifera* tend to be highly susceptible to crown gall, although certain French-American hybrids and native-American varieties may also become severely infected (Sule et al., 1994).

Studies showed that *A. vitis* can survive systemically in vines, and thus affect subsequent vascular tissue development, block nutrient and water flow, reduce vine vigor, and cause substantial economic loss for grape growers, nurseries, and product producers (Burr et al., 1998; Cubero et al., 2006). *A. vitis* can remain viable in small pieces of dead grapevine debris in the soil for at least 2 years after removal or death of a vine, while other researchers have found that grape roots may remain viable in the soil for at least 5 years after the removal of a vine (Burr et al., 1995).

Several methods such as avoiding plant infected material in the vineyard and grafting to resistant rootstocks have been used in controlling grapevine crown gall disease (Burr and Otten, 1999). Biological control agent K84 works only on some plant species such as peach, and only effective in controlling crown gall caused by nopaline-type strains of *A. tumefaciens*, but has no effect on crown gall induced by *A. vitis* in grapevines (Burr et al., 1998; Kawaguchi et al., 2005). With no effective available chemical control, the most important step in preventing crown gall disease in grapevine is to plant crown gall disease resistant grapevines.

Extensive researches have been conducted in determining the disease resistance of *V. vinifera* and their derivative grapes (Burr and Otten, 1999). No information is available for muscadine grape cultivars. The objectives of this research were to screen the crown gall tolerant/resistant genotypes and rootstocks in green and dormant cuttings of muscadine grapes (*V. rotundifolia* Michx.)

Material and Methods

Plant material. Green and dormant cuttings from 29 commercially available muscadine grape cultivars, five Florida hybrids, five *V. vinifera*, two French-American hybrids, and *Shuttlewuthii* sp. obtained from grape germplasm repository or PD free cage at the Florida A&M University, Tallahassee, were used for screening

Acknowledgments. This research is supported in part by the Viticulture Advisory Council, FDACS.

*Corresponding author; phone: (850)412-7393; email: jiang.lu@famu.edu

the resistance to crown gall disease (Table 1). Cuttings originated from 6- to 7-year-old vines without visible symptoms of crown gall were collected in June and December, respectively, and used for the pathogen inoculation. About 25- to 30-cm-long and 5- to 7-mm-diameter old cuttings were used for pathogen inoculation.

***Agrobacterium* strain.** *Agrobacterium* strain *vitis* C493 and C4612 was used for inoculation and gifted by Dr. H.K. Yun from the National Horticultural Research Institute. The strain was isolated from infected grapevine stem and was maintained at –80 °C in a 30% glycerol stock. Three days prior to inoculation, the pathogen was streaked on a potato dextrose agar (PDA, 24 g·L^{-1} potato dextrose broth + 20 g·L^{-1} agar) and grown at 28 °C. The single colony was then transferred into a yeast–beef extracts–peptone (YEP) medium [Yeast extract 1 g, (beef extract 5 g, peptone 5 g), sucrose 5 g, $MgSO_4$ 0.5 g·L^{-1}, pH 7.2] and allowed to grow at 28 °C in a shaking incubator (140 rpm) for 18 h (Sule et al., 1994). The bacterial cells were collected by centrifugation at 3000 rpm for 15 min, resuspended in sterile distilled water and adjusted to 109 cfu/mL (OD600 = 1.0) for inoculation ("admincharley" caps lock on for Nonodrop, program ND-1000V 3.3.0, cell culture, 1 µL solution).

Table 1. Scoring of crown gall inoculated by C493 and C4612.

Cultivar name	C493 (g)[z]	C4612 (g)[z]
Africa Queen	0.0375	0.2338
Alachua	0.2375	0.1475
Albemarle	0.0563	0.1650
Black Beauty	0.0413	0.0825
Black Fry	0.0538	0.0850
Carlos	0.0225	0.0300
Cowart	0.0400	0.1625
Darlene	0.0613	0.3675
Digby	0	0.1313
Dixie	0.0600	0.1463
Dixie Land	0.0888	0.1138
Dixie Red	0.0388	0.0950
Doreen	0.0225	0.1150
Fry	0.0088	0.0725
Farrar	0.1288	0.1600
Florida Fry	0.0463	0.0638
Fry	0.0125	0.1138
Fry Seedless	0.1075	0.1188
Gold Isle	0.0863	0.2550
GranyVal	0.0013	0.0650
Higgins	0.0050	0.0625
Hunt	0.0250	0.0475
Ison	0.0363	0.1513
Jumbo	0.0325	0.0488
Later Fry	0.0238	0.1013
Nesbitt	0.0700	0.1263
Noble	0.0125	0.0213
Supreme	0.0563	0.0225
Triumph	0.0450	0.1163
Black Spanish	0	0
Blanc du Bois	0	0
Conquistador	0	0
Orlando Seedless	0	0
Stove	0	0
Cabernet Sauvignon	0.0031	0.0063
Chardonnay	0.0119	0.0188
Chenin Blanc	0	0.0019
Merlot	0.0013	0
Thompson Seedless	0	0
Hunnish × Triumph	0.0075	0.0138
Jumbo × Thompson Seedless	0.0025	0.0044
Shuttlewuthii	0	0.0025

[z]Data were the average of total weight (inoculated sites + cutting tops) in eight inoculated cuttings with four replications per treatment.

Inoculation. For the pathogen inoculation, bacterial suspensions (30 µL) kept on ice were injected into the holes (3 mm in diameter) drilled on internodes. Inoculated sites were wrapped with parafilm and were maintained in mist beds or water in growth room. Water was changed every 3 d.

Scoring gall formation. Two months after pathogen inoculation, inoculated sites and cutting side of the top were scored for gall formation. Each plant was scored for incidence rate of galls as well as the gall weight. Electromicroscope analyses were also used for the confirmation of the appearance of the bacteria.

Results and Discussion

Two months after the inoculation and growth in mist beds, abnormal overgrowth was observed on sites of nine muscadine grape cultivars inoculated with *A. vitis* C493, and more abnormal overgrowth was found on top sites of the green cuttings. Cultivar Digby was free of abnormal overgrowth either on inoculated sites or on top sites of the cuttings when inoculated with *A. vitis* C493 (Table 1), although overgrowth was observed for cuttings inoculated with *A. vitis* C4612. All muscadine cultivars showed abnormal overgrowth either on inoculated sites or on top sites of the cuttings. The average gall size ranged from 0.0213 to 0.3675 g, which was larger than those on cuttings inoculated with C493. As expected, larger levels of abnormal overgrowth occurred on green cuttings inoculated with the C4612. Out of 29 muscadine grape cultivars, 27 showed abnormal overgrowth on inoculated sites and 25 cultivars had abnormal overgrowth on top sites of the cuttings. Interestingly, no abnormal overgrowth was found in five Florida hybrids, and less degree of abnormal overgrowth appeared in *V. vinifera*, French-American hybrids, and *Shuttiwuthii* sp.

Similar results were obtained for dormant cuttings of muscadine grapevines (Fig. 1). However, only inoculated cuttings from *V. vinifera* and Florida hybrid bunch grapes showed callus/gall growth for C493 and C4612 that ranged from about 0.2 to 0.3 g at inoculated sites. No callus/gall growth was noticed for controls. Scanning electron micrograph analysis of the growth tissue found the presence of bacteria in inoculated cuttings but not in controls for all cultivars (Fig. 2).

Appearance of the abnormal overgrowth on muscadine grapevine cuttings is in contradiction to the general concept about crown gall disease resistance in *Vitis* sp. It seems that muscadine grapes are not completely immune to *A. vitis* infection. The difference in crown gall disease resistance between *Vitis* sp. can only be explained by their developmental stage since cuttings at the same time during a growing season may reflect different stages. This is normally true that muscadine grapevines bloom and mature 2 months later than other species. Comparison in crown gall disease between species can only be made when the cuttings are obtained at the same stage of biological development.

Literature Cited

DeCleene, M. and J. DeLey. 1976. The host range of crown gall. Bot. Rev. 42:389–466.

Burr, T.J. and B.H. Katz. 1984. Grapevine cuttings as potential sites of

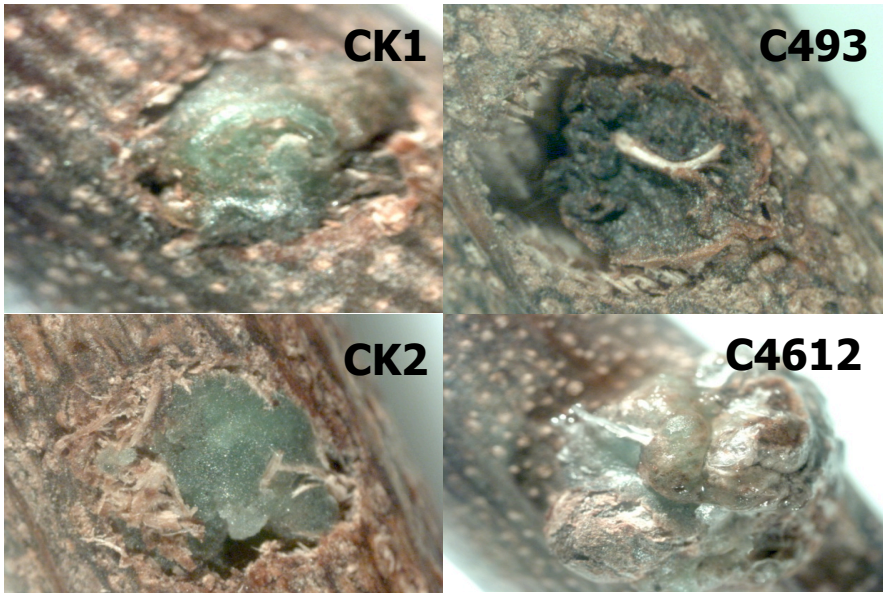

Fig. 1. Results of dormant cuttings of 'Higgins'.

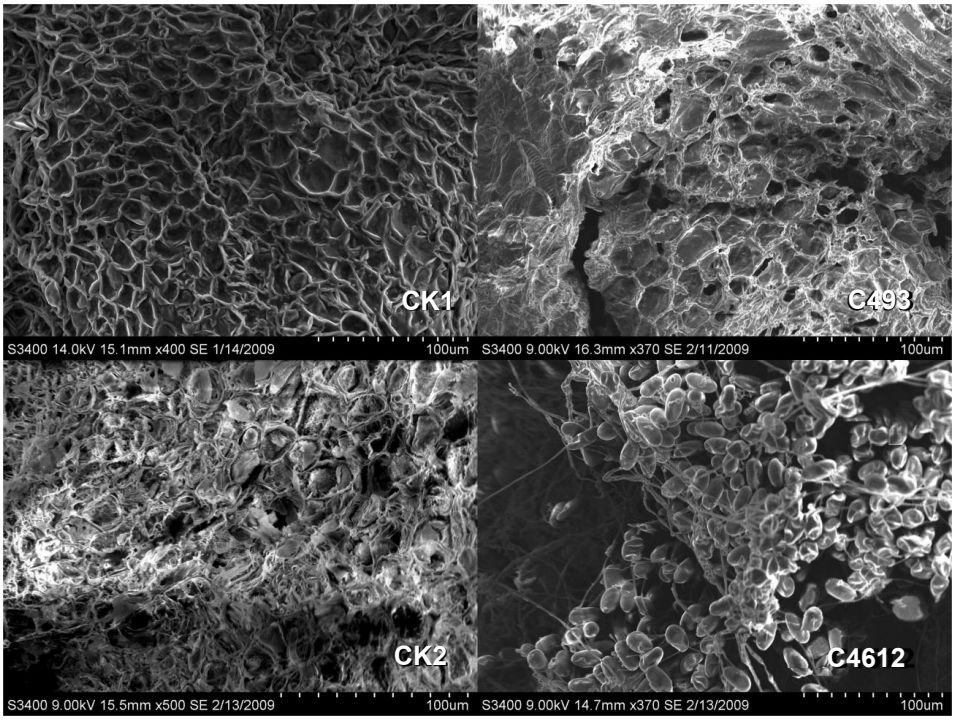

Fig. 2. Results of electron microscopic scanning for dormant cuttings of 'Higgins'.

survival and means of dissemination of *Agrobacterium tumefaciens*. Plant Dis. 68:976–978.

Burr, T.J., C. Bazzi, S. Sule, and L. Otten. 1998. Crown gall of grape: Biology of *Agrobacterium vitis* and the development of disease control strategies. Plant Dis. 82:1288–1297.

Burr, T.J. and L. Otten. 1999. Crown gall of grape: Biology and disease management. Annu. Rev. Phytopathol. 37:53–80.

Burr, T.J., C.L. Reid, M., Yoshimura, E.A. Monol, and C. Bazzi. 1995. Survival and tumorigenicity of *Agrobacterium vitis* in living and decaying grape roots and canes, in soil. Plant Dis. 79:677–682.

Cubero, J., B. Lastra, C.I. Salcedo, J. Piquer, and M.M. Lopez. 2006. Systemic movement of *Agrobacterium tumefaciens* in several plant species. J. Appl. Microbiology 101:412–421.

Kawagychi, A., K. Inoue, and H. Nasu. 2005. Inhibition of crown gall formation by *Agrobacterium radiobacter* biovar 3 strains isolated from grapevine. J. Gen. Plant Pathol. 71:422–430.

Miller, J. 1988. Crown gall of woody plants in Florida. Plant Pathol. Circ. No. 305.

Ophel, K. and A. Kerr. 1990. *Agrobacterium vitis* sp. Nov. for strains of *Agrobacterium tumefaciens* biovar 3 from grapevines. Intl. J. System. Bacteriol. 40:236–241.

Sule, S. and T.J. Burr. 1998. The influence of rootstock resistance to crown gall (*Agrobacterium* spp.) on the susceptibility of scions in grape vine cultivars. Plant Pathol. 47:84–88.

Sule, S., J. Mozsar, and T.L. Burr. 1994. Crown gall resistance of *Vitis* species and grapevine rootstocks. Phytopathology 84:607–611.