Grapevine Cuttings as Potential Sites of Survival and Means of Dissemination of *Agrobacterium tumefaciens*

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ABSTRACT

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Agrobacterium tumefaciens (AT) biovar 3 and (to a lesser extent) biovar 1 were detected in symptomless 1-yr-old grape cane cuttings taken from crown gall-infected vines, thus indicating their systemic nature. With selective media, both biovars were isolated from callus and small roots of surface-sterilized cuttings rooted in a sterilized potting mixture. Pathogenic strains were recovered from the cultivars Gewurtztraminer, Pinot Chardonnay, Pinot Noir, and White Riesling. The pathogen was not detected in canes taken from apparently healthy vines. AT biovar 3 was recovered from one of 10 soil samples collected around trunks and roots of vines with crown gall symptoms.

Recent reports indicate that *Agrobacterium tumefaciens* (AT) biovar 3 is found predominantly on grapevines (4–6,10,15,20,21,26) and that the bacterium can be detected in sap collected from "bleeding vines" (5,12). Our previous findings (5) and those of Lehoczky (12,13) indicate that the bacterium survives systemically in grapevines. Limited assays of soil have resulted in recovery of widely varied ratios of pathogenic to nonpathogenic agrobacteria (5,7,8,14,24,25). Results presented in this paper indicate that AT can be present in dormant grape cuttings that may be used in propagation of plants. We also question the relative importance of soil versus systemic inoculum for inciting severe crown gall outbreaks on grapevines. This paper confirms the systemic nature of AT in dormant grape cuttings and the relatively low recovery rate of the pathogen from soil.

MATERIALS AND METHODS

Dormant cutting assay. Dormant cane cuttings were collected from two vineyards and one nursery in the Finger Lakes region of New York State during February of 1982 or 1983. In 1982 samples were taken from eleven 12-yr-old crown gall-infected vines: two Gamay Beaujolais, one Gewurtztraminer, four Pinot Chardonnay, two Pinot Noir, and two White Riesling. In 1983, cuttings consisting of the cultivars Cabernet Sauvignon, Pinot Chardonnay, Pinot Noir, Sauvignon Blanc, and White Riesling were obtained from a commercial nurseryman. These samples, designated 1983A, came from various vineyards in New York and other states and the conditions of the mother vines with respect to crown gall were not known. Samples were also collected from a 10-yr-old commercial vineyard (1983W) that a nurseryman used as a source of propagation material. Samples from five infected and five apparently uninfected vines of Pinot Chardonnay and White Riesling were collected. From the vines sampled in 1982 and 1983, four to 30 cuttings were collected for assay from each.

Three-node cuttings were prepared from each sample and surface-sterilized in a 0.53% NaOCl solution for 3 min. Cuttings were then rinsed in tap water and 1 cm was cut from each end to remove any residual NaOCl. Sterilized cuttings were rooted on a heated greenhouse bench in a sterile potting medium of sand, Cornell mix (3), or vermiculite. Four to 7 wk later, isolations were attempted from the callus and/or small roots. The lower half of the cutting was soaked in 0.53% NaOCl for 3 min, then rinsed in tap water for 5 min. Specific tissues were removed from the cutting with a sterile scalpel and triturated in 2 ml of sterile distilled water (SDW) with a mortar and pestle. Triturate was streaked on modified New and Kerr medium (NKS) (5) and incubated at 28 C for 4 days. Colonies resembling AT were transferred to potato-dextrose agar (Difco) (PDA) to verify colony morphology and to prepare inoculum for pathogenicity tests. Triturates from the 1983W samples were additionally streaked on a medium (RS) recently developed for biovar 3 by Roy and Sasser (22).

Pathogenicity tests. We reported previously that strains of biovar 3 collected in the Finger Lakes region of New York State have a broad host range and more than 90% are pathogenic on sunflower (*Helianthus annuus* L.) (5). In this study, inoculum was grown for 3 days on PDA, then bacterial growth was stab-inoculated with a sterile needle into the lower stems of 1-wk-old sunflower plants. Three to six plants were inoculated per strain and final pathogenicity readings were taken after 3 wk.

Soil and gall isolations. In September 1983, current-season galls and soil were collected from vineyard 1983W. Galls were collected from six Pinot Chardonnay and four White Riesling vines, placed in glass vials, and transported to the laboratory. Three soil probes, each about 10 cm deep, were collected beneath each vine, combined in a plastic bag, and taken to the laboratory.

Isolations from galls were done as described previously (5), except platings were made on all the following media: Schroth et al (SCH) (23), NK (18), RS, and NKS. The 10 soil samples were each mixed thoroughly to combine soil from the three probes. Subsequently, 10-g subsamples were stirred in 100 ml of SDW for 20 min. Serial dilutions in SDW were then plated on each of the four selective media. A total of 219 strains were collected from AT-like colonies (138 from galls and 81 from soil) and tested for pathogenicity as described previously.

Characterization of strains. When pathogenicity was confirmed, a representative number of strains from each cutting and from vineyard soil were identified to biovar using the following criteria: growth on SCH, NK, and RS media; production of 3-ketolactose (2); growth in 2% NaCl (17); action on litmus milk (10); acid production from erythritol and melezitose (10); and alkali production from malonate (10). In addition, strains from the 1982 samples were inoculated to sunflower, tomato (*Lycopersicon esculentum* Mill. cv. New Yorker), and grapevine (*Vitis vinifera* L. cv. White Riesling) in the greenhouse. All tests were repeated at least twice and included previously identified strains of biovars 1, 2, and 3 as controls.

RESULTS

Isolations from dormant cuttings. Pathogenic AT strains were obtained

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from five of the 11 vines sampled in 1982 (Table 1), including one vine each of Pinot Noir and Gewurtztraminer and three vines of Pinot Chardonnay. None were detected in Gamay Beaujolais or White Riesling vines. Of 148 AT-like strains inoculated to sunflower, 65 (44%) were pathogenic. Fourteen strains (at least one from each cutting from which pathogenic strains were recovered) were identified as biovar 3 (Table 2). Almost all the strains were pathogenic on sunflower (13/14), tomato (14/14), and grapevine (11/14) and all were pathogenic on at least two of the three hosts.

Pathogenic AT strains were obtained from four of the seven vines from the 1983A sample, including one Pinot Noir and three White Riesling (Table 1). None were detected from the cultivars Sauvignon Blanc, Pinot Chardonnay, or Cabernet Sauvignon. Of 153 AT-like strains inoculated to sunflower, only 13 (9%) were pathogenic. All 13 were characterized as biovar 3 (Table 2).

Cuttings taken from White Riesling and Pinot Chardonnay vines showing crown gall symptoms in vineyard 1983W yielded pathogenic AT, whereas cuttings from apparently healthy vines did not (Table 1). Of 240 possible candidate strains inoculated to sunflower, 53 (22%) were pathogenic and were identified to biovar (Table 2). Seven (recovered from NKS medium) were characterized as biovar 3, and 46 (six from NKS plates and 40 recovered from RS) were biovar 1. All seven biovar 3 strains were obtained from one White Riesling cutting.

Soil and gall isolations. Recoveries of AT were made from White Riesling and Pinot Chardonnay galls and from soil collected beneath a Chardonnay vine. Most pathogenic strains were recovered on RS and NKS media, fewer on NK medium, and none on SCH. Although nine of 10 gall samples yielded AT, only one of 10 soil samples was positive. Of 138 strains from galls inoculated to sunflower, 97 (70%) were pathogenic (58 from White Riesling and 39 from Pinot Chardonnay). Eighty-one AT-like strains from soil were inoculated to sunflower and four (5%) were pathogenic.

Thirty-four pathogenic strains obtained from the soil and gall isolations were identified as biovar 3 (Table 2), regardless of the medium from which they were initially recovered. In order to positively identify AT strains as opposed to *A. radiobacter*, it is necessary to conduct pathogenicity tests. In this study, we ran pathogenicity tests on a total of 760 suspect AT strains. The percentage that were pathogenic ranged from 5 to 70% and varied greatly among samples.

DISCUSSION

Systemic movement of AT has been reported previously in chrysanthemum (16). Although some researchers failed to isolate AT from grapevine sap (6), results from this study and previous research (5,12,13) clearly demonstrate its systemic nature. We have sampled relatively few cutting sources; however, it appears that AT-contaminated cuttings may be a major problem for the grape nursery industry.

Reliable methods for preventing and eradicating crown gall of grapevine are not available. Removing infected trunks and canes and bringing up renewals helps manage the disease in some vineyards, but it is not a cure. Considering the systemic nature of the pathogen, it is not surprising that chemical controls with nonsystemic bactericides have not provided satisfactory control (14). Biological control with *A. radiobacter* strain 84 also has not been effective (20; T. J. Burr and B. H. Katz, *unpublished*).

Our results indicate that one approach nurserymen should use to reduce the risk of propagating vines with high systemic populations of the pathogen is to propagate only from healthy vines. Because infected trunks and canes are usually removed during pruning operations, however, it may be impossible to tell if a vine has had crown gall and is therefore possibly harboring the pathogen. The detection limit of the method used in this study is unknown and even some of the apparently healthy vines may harbor undetectable populations of AT.

The detection method used in this study is suitable for demonstrating the presence of the pathogen in a small number of samples but may not be practical for use on a commercial scale. The entire procedure may take 6 wk and

Table 1. Recovery of *Agrobacterium tumefaciens* (AT) from callus and roots of grapevine cuttings[a]

Sample	Cultivar (no. vines)	No. cuttings from which AT recovered/ no. sampled	No. strains of AT recovered/ no. tested on sunflower[b]
1982	Pinot Noir (2)	1/7	8/25
	Gamay Beaujolais (2)	0/4	0/0
	Gewurtztraminer (1)	1/2	9/12
	Pinot Chardonnay (4)	6/17	48/100
	White Riesling (2)	0/7	0/11
Total		8/37	65/148
1983A	Pinot Noir (1)	1/13	3/32
	White Riesling (3)	13/62	10/62
	Sauvignon Blanc (1)	0/11	0/13
	Pinot Chardonnay (1)	0/20	0/34
	Cabernet Sauvignon (1)	0/14	0/12
Total		14/120	13/153
1983W	Crown gall-infected		
	White Riesling (5)	5/15	42/54
	Pinot Chardonnay (5)	3/15	11/62
Total		8/30	53/116
	Apparently healthy		
	White Riesling (5)	0/15	0/81
	Pinot Chardonnay (5)	0/15	0/43
Total		0/30	0/124

[a] Cuttings were rooted in autoclaved sand, vermiculite, or Cornell mix (3). Four to 7 wk later, callus and roots were surface-sterilized, excised, triturated in water, and streaked on the selective media, NKS (5) or RS (22). Suspect AT strains were inoculated to sunflower to test for pathogenicity.
[b] Strains were selected for pathogenicity tests on the basis of colony morphology and growth characteristics on selective media and potato-dextrose agar.

Table 2. Characterization of *Agrobacterium tumefaciens* (AT) strains from grapevine cuttings, galls, and vineyard soil

Test	AT biovars[a]			Cuttings			Galls	Soil
	1	2	3	1982	1983A	1983W		
Growth on SCH medium	+	−	−	0/14[b]	0/13	46/53	0/30	0/4
Growth on NK medium	−	+	−	0/14	0/13	0/53	0/30	0/4
Characteristic growth on RS medium[c]	−	−	+	8/8	13/13	5/53	30/30	4/4
Production of 3-ketolactose	+	−	−	0/14	0/13	46/53	0/30	0/4
Growth in 2% NaCl	+	−	+	14/14	13/13	53/53	29/30	4/4
Litmus milk	Alk.	Acid	Alk.	Alk.	Alk.	Alk.	Alk.	Alk.
Acid from erythritol	−	+	−	0/14	0/13	0/53	0/30	0/4
Acid from melezitose	+	−	−	0/14	0/13	46/53	0/30	0/4
Alkali from malonate	−	+	+	14/14	9/13	4/53	24/30	3/4

[a] Results of characterization tests for AT biovars 1, 2, and 3 are from Moore et al (17), except for growth on RS (22) medium, which was conducted in our laboratory.
[b] Number of positive strains divided by the total number of strains tested.
[c] Biovars 1 and 2 may grow on RS but more slowly than biovar 3.

requires many hours for isolation and identification of pathogenic strains. One improvement used in a portion of this research is RS medium, which is highly selective for biovar 3 and eliminates many contaminants that grow on NKS. We have found, however, that biovars 1 and 2 may grow on RS but more slowly than biovar 3. Attempts are currently under way, using RS, to isolate the pathogen directly from dormant cuttings. Even if we are successful, however, it will still be necessary to identify the pathogenic strains using greenhouse pathogenicity tests. Other methods of identifying virulent strains of AT have recently been discussed by Kerr and Brisbane (9) and may prove practical in the future.

The taxonomy of AT from grapevines needs further elucidation. Although it has been reported in several areas of the world that biovar 3 is the predominant biovar from grapevines (4–6,10,15,20, 21,26), major differences among strains are apparent. For example, the strains from Greece are reported to have a very narrow host range (19,20) in contrast to strains from Hungary, Canada, and the United States, which have broad host ranges (5,6,21,26). In addition, close examination of plasmids from biovar 3 strains have identified genetic differences among strains (11,21). Although biovar 3 is considered a grapevine pathogen, it has also recently been reported on chrysanthemum (1).

It is apparent that every cutting from a vine may not harbor pathogenic AT strains. Either the bacterium is not evenly distributed within canes or populations in many samples were too low for successful detection by our method. Also, a high proportion of avirulent, presumably *A. radiobacter*, strains were recovered from many canes.

When biovar tests were performed, most isolates identified as biovar 3 conformed to previous results obtained with known biovar 3 isolates, except for the variable response obtained in alkali production from malonate.

Interestingly, our 1983W sampling of cuttings yielded predominantly biovar 1 strains. When we sampled the same vineyard during the growing season, the galls and soil samples yielded biovar 3. Although we have no explanation for this inconsistency, evidence from previous research shows that strains other than biovar 3 can be detected occasionally from grapevine crown gall (5,20).

Limited soil samplings reported here and in a previous study (5) show that recovery of AT was successful from vineyard soil near the trunks of infected vines. Samples taken between rows thus far have not yielded the pathogen (*unpublished*). The low number of pathogenic strains recovered from soil agrees with results from our previous research (5). It appears, therefore, that AT biovar 3 may not survive at high populations in vineyard soil. Compared with systemic inoculum present in propagation material, soil may be a minor source of inoculum.

It will now be important to develop a means of eliminating AT from grape plant material. Although the extent of systemic contamination in different cultivars or regions is not known, it is probably substantial. Some vines may become contaminated in the vineyard from the low level of soil inoculum, but by planting AT-free vines, devastating incidences of crown gall on 2- to 3-yr-old vines may be prevented. If sources of AT-free propagation material become available to nurserymen, it will be possible to produce certified AT-free grapevines. Currently, we are attempting to free cultivars of the pathogen by meristem tip culture.

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