Crown Gall Resistance of *Vitis* spp. and Grapevine Rootstocks

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ABSTRACT

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Vitis riparia 'Gloire de Montpellier' (Gloire) was the most resistant *Vitis* species and clone tested following inoculations with strains of *Agrobacterium vitis* and *A. tumefaciens*. Although tumors failed to appear after infection, the pathogen *A. vitis* multiplied at the same rate in Gloire as in the crown gall-susceptible *V. vinifera* 'Chasselas.' Root decay was induced by strains of *A. vitis* on all the investigated *Vitis* genotypes. Similarly, roots and shoots of all *Vitis* genotypes produced *vir* gene-inducing compounds. This fact ruled out the possibility that crown gall resistance is related to the lack of *vir* gene induction. However, T-DNA from *A. vitis* strain CG49 transferred to *V. riparia* cells at a lower rate than to *V. vinifera* cells, as determined by evaluation of *uid*A gene (GUS) expression.

Additional keywords: grape, opines.

Crown gall of grapevine is caused by the bacterial pathogen *Agrobacterium vitis* (13), formerly *A. tumefaciens* biovar 3, and by *A. tumefaciens* biovar 1. *A. vitis* strains have been isolated from grapevine (*Vitis vinifera* L.), and several studies (2,13–15,20) have shown the close association between the pathogen and this host plant. *A. tumefaciens* biovar 1 is a polyphage organism infecting many dicotyledonous plants, especially fruit trees. Both *Agrobacterium* species can induce tumors on grapevine, although their

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infection processes probably are different (2,9). *A. vitis* produces polygalacturonase (9), which is associated with a decay of grapevine roots (2). The bacteria may enter the roots through root lesions caused by *A. vitis* and systemically infest the whole plant. The infection process of *A. tumefaciens* biovar 1 on grapevine has not been studied. However, the biovar 1 strain can be isolated from grapevine tumors and will cause tumors following inoculation into artificial wounds. The host range determined by the Ti plasmid of *A. vitis* is wide for some strains and narrow for others (8). *A. tumefaciens* biovar 1 strains isolated from grapevine have a wide host range, but many of them have special characters, such as the ability to utilize tartrate (20). Genetic studies of *A. vitis* show that this species is rather diverse (15). It can be divided into strains with nopaline, octopine-cucumopine, and vitopine Ti plasmids (15,21), and in each group several subgroups exist. The octopine-cucumopine strains have Ti plasmids with two transferred DNA (T-DNA) regions, TA and TB. On the basis of the TA region, they can be divided (14) into strains having small TA regions, e.g., Ag57, Ag162, and AB3 (strains with limited host ranges), or large TA regions, e.g., Tm4 and K305 (strains with wide host ranges). At the site of infection, the bacterium transfers a part of its Ti plasmid DNA (T-DNA) to plant cells. The T-DNA encodes auxin and cytokinin production by plant cells. A different region of the Ti plasmid, the virulence (*vir*) region, regulates the transfer (10). Together the T region and the *vir* region can determine tumorigenesis of the bacterium, but sensitivity of genotypes to plant hormones may also be associated with tumor formation. Different *Vitis* spp. often respond differently to infection by *Agrobacterium* spp. Cultivars of *V. vinifera* are susceptible, whereas some *V. amurensis* clones and their crosses are resistant (22).

Avirulence of the bacterium or resistance of the plant can be caused by either a lack of T-DNA transfer or a failure of some subsequent step leading to symptom formation. In this study, we evaluated the resistance of *Vitis* genotypes to crown gall. Different strains of *A. vitis* and *A. tumefaciens* were inoculated on different *Vitis* spp., and evaluations were made of tumor weights, opine synthesis, appearance of root decay, multiplication of bacteria at inoculation sites, *vir* gene induction, and level of T-DNA transfer.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. Table 1 lists the strains used in this study. Bacteria were cultivated on yeast extract-beef extract-peptone (YEB) medium (1 g of yeast extract, 5 g of beef extract, 5 g of peptone, 5 g of sucrose, and 0.5 g of $MgSO_4$ in 1 L of distilled water) for 48 h at 25 C and suspended prior to inoculation in sterile distilled water. For the *vir* induction assay, a modified AB medium (24) was used. Plasmid p35SGUSINT (23) was introduced into CG49 from *Escherichia coli* strain S17-1. Minimal medium amended with trimethroprim (50 mg/L) was used to counterselect against *E. coli*.

Plant material and infections. The *V. riparia* clones used had been maintained in a collection in Hungary since the end of the last century when they were imported from the United States for a phylloxera breeding program. Some rootstocks currently used in Hungary and Paulsen 775, which had been reported to

TABLE 1. Origin and characteristics of *Agrobacterium* strains and plasmids used in this study

Strain	Host range	Opine	Plasmid	Origin
A. vitis				
2/3	Wide	Nopaline	pTi(wilde)	S. Süle, Hungary
2/3S5R(Sm^R,Rp^R)[z]	Wide	Nopaline	pTi(wilde)	Derivative of 2/3
CG49	Wide	Nopaline	pTi(wilde)	T. J. Burr, United States
AT-1	Wide	Nopaline	pTi(wilde)	J. Lehoczky, Hungary
AB3	Limited	Octopine	pTi(wilde)	E. Szegedi, Hungary
Tm4	Wide	Octopine	pTi(wilde)	E. Szegedi, Hungary
Ag162	Limited	Octopine	pTi(wilde)	P. G. Psallidas, Greece
A. tumefaciens biovar 1				
16/6	Wide	Nopaline	pTi(wilde)	S. Süle, Hungary
1/21	Wide	Nopaline	pTi(wilde)	I. Popova, Bulgaria
A348	Wide	...	pSM358 *virE::lacZ*	E. W. Nester, United States
C58	Wide	...	p35SGUSintron	L. Willmitzer, Germany

[z] Sm^R = streptomycin resistant, and Rp^R = rifampicin resistant.

TABLE 2. Sensitivity of grapevine genotypes to crown gall induced by different strains of *Agrobacterium*

Vitis sp. Cultivar	*A. tumefaciens*[y] 16/6	*A. tumefaciens*[y] 1/21	*A. vitis*[y] AT-1	*A. vitis*[y] AB3	*A. vitis*[y] Tm4	*A. vitis*[y] 2/3	*A. vitis*[y] CG49	*A. vitis*[y] Ag162	Total[z]
V. riparia									
Gloire	0.0 f	0.0 f	0.0 e	11.0 cd	4.0 d	0.0 e	0.0 e	0.0 f	15.0
Gloire K	0.0 f	0.0 f	0.0 e	16.3 cd	0.0 d	0.0 e	0.0 e	0.0 f	16.3
selecta	24.3 ef	0.0 f	32.0 cde	5.7 cd	19.7 cd	3.7 e	9.7 e	0.0 f	95.1
sauvage	0.0 f	0.0 f	40.3 cd	0.0 d	0.0 d	0.0 e	27.7 de	29.3 bcd	97.3
tomentosa	26.0 ef	13.7 ef	48.0 cd	32.3 bcd	30.0 cd	25.7 de	0.0 e	9.3 def	185
Martin	14.0 ef	36.3 def	32.0 cde	22.7 bcd	10.7 d	22.7 de	47.7 de	0.0 f	186.1
V. riparia × *V. cordifolia*	60.0 cd	55.0 cd	39.3 cd	35.7 bcd	11.0 d	13.7 de	33.7 de	36.0 bcd	284.4
V. riparia × *V. rupestris*									
287C	98.0 b	105.0 b	69.0 c	24.0 bcd	40.3 cd	41.7 cd	35.3 de	34.7 bcd	448
101-14Mgt	44.7 de	40.3 de	20.3 de	0.0 d	0.0 d	0.0 e	18.7 e	5.7 ef	129.7
V. berlandieri × *V. rupestris* Paulsen 775	22.3 ef	28.7 def	18.3 de	46.0 bc	66.7 bc	98.0 b	209.3 b	22.7 cdef	512
V. berlandieri × *V. riparia*									
Teleki 5BB	81.3 bc	54.0 cd	108.3 b	143.3 a	53.0 cd	76.3 b	80.0 cd	47.3 bc	643.5
Teleki 8B	67.7 bcd	89.7 bc	56.7 cd	66.0 b	111.7 b	102.0 c	102.0 c	54.7 b	650.5
Teleki 5C	71.2 bcd	92.0 bc	58.0 cd	65.8 b	122.0 b	112.0 c	98.0 c	62.0 b	681
V. vinifera									
Chasselas	279.3 a	183.3 a	317.3 a	173.7 a	201.7 a	227.3 a	349.7 a	93.0 a	1,825.3

[y] Values represent the average weight of tumors in milligrams from three repetitions. Means with the same letter are not significantly different at $P = 0.05$ according to the Waller-Duncan K ratio t test.
[z] Not statistically compared.

be immune to all *A. vitis* strains (3), were also included in the investigations. The *Vitis* spp. used in this study are listed in Table 2.

Grape cuttings were collected in February from the collection of the Pannon University at Keszthely in Hungary. They were cut in two-bud lengths and planted in pots containing sterilized soil. The greenhouse temperature was 18–28 C. When the new shoots were 25–30 cm long, they were inoculated at the third and fourth internodes by making wounds (2 × 2 × 2 mm) with a lance and depositing 10 μl of a bacterial suspension containing about 10^{10} cfu/ml into each wound. Plants were kept at 18 C for 48 h after inoculation to facilitate T-DNA transfer and then moved to the greenhouse. Each plant-bacterium combination was repeated at least four times. Tumors were weighed after 2 mo.

Growth of *A. vitis* in plants. Four petioles per plant, located 25–30 cm above the base of each vine, were wounded with a scalpel and inoculated with a suspension containing about 10^8 cfu of *A. vitis* strain 2/3S5R per milliliter. Wounds (about 1–2 mm^2 and 0.5 mm deep) were made at approximately the middle of each petiole. Two microliters of bacterial suspension was applied from a Hamilton micropipette to each wound. Inoculated petioles were removed from designated plants 2 h and 2, 4, 6, and 8 days after inoculation. A 1-cm section of each petiole surrounding the inoculation point was removed, and four individual samples were collectively ground in 1 ml of sterile distilled water in a mortar. Bacterial populations were assessed by standard dilution plating techniques on YEB medium.

Root decay assays. Dormant cuttings of grape canes were planted in perlite, and the new white roots were harvested after 1–2 mo. Roots were surface sterilized with 0.1% sodium hypochloride and rinsed in sterile distilled water three times. Root pieces approximately 2–3 cm long were placed on moist filter paper in petri dishes and wounded by making a single needle puncture in the middle of each root piece. All the roots were then inoculated by depositing a 3-μl drop of bacterial suspension (about 10^{10} cfu/ml) on each wounded region. The petri dishes were kept at 25 C for 4–5 days, and the appearance of necrosis was assessed with a dissecting microscope (4×). Four wounded root pieces were inoculated for each strain. Sterile water served as the control.

Determination of opines. Galls, swellings, or the surrounding tissues of the wound sites were excised and cut into small pieces. Extracts were prepared by grinding tissues in a minimum amount (1:1 [w/v]) of 70% ethanol with a small pestle in microcentrifuge tubes. The extract (5–10 μl) was spotted onto Whatman 3MM paper, electrophoresed, and visualized as described (17).

Assay of *vir* gene induction. Strain A348(pSM358) carrying a *virE::lacZ* fusion was employed to monitor the induction of the *vir* genes in tissue culture-grown shoot and root pieces of Narancsizü (*V. vinifera*) and Gloire. Plants were grown in MS (Murashige-Skoog [12]) medium supplemented with 0.1 mg of α-naphthaleneacetic acid per liter. When the shoots were 30–40 mm long, plants were removed from the culture tubes and defoliated. Shoots and roots were cut into sections 10 mm in length.

Bacteria were precultivated for 48 h in low-phosphate AB sucrose medium optimized for *vir* induction assays (24). The medium contained 3% sucrose, AB salts, 2.5 mM phosphate buffer, 20 mM MES (morpholineethanesulfonic acid), and 50 mg each of ampicillin and kanamycin per liter. This medium was adjusted to pH 5.5 for *vir* gene induction. The bacteria were suspended in the same liquid medium (10^8 cfu/ml) without antibiotics. They were spread on the surface of the above medium (24) containing 1% agar, 20 mg of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) per milliliter, and 20 mg of IPTG (isopropyl-β-D-thiogalactopyranoside) per liter. Shoot and root pieces were laid on the surface of this medium. The appearance of blue color at the ends of pieces was monitored 2–3 days later. Leaf petiole segments (10 mm long) of other *Vitis* spp. were collected from greenhouse-grown plants, surface sterilized, and assayed in the same manner as were the plant materials grown in vitro.

Assay for T-DNA transfer. Bacterium-free shoot sections (10 mm long) grown in vitro were inoculated at the apical ends with a 2-μl suspension (10^{10} cfu/ml) of strain CG49(p35SGUSintron). The explants were kept for 24 h between moist filter papers and then put in MS medium without hormones with their basal ends inserted into the agar. After 3 days, the in vivo GUS (β-glucuronidase) assay (1,7) was performed. The apical ends of inoculated segments were put into Eppendorf tubes containing 100 mM sodium phosphate, pH 7.0, 10 mM EDTA, 0.5 mM $K_3Fe(CN)_6$, 0.5 mM $K_4Fe(CN)_6 \cdot 3H_2O$, 0.01% Triton X-100, and 2.0 mM X-Gluc (5-bromo-4-chloro-3-indolyl β-D-glucuronide; Sigma Chemical Co., St. Louis, MO). Tissues were vacuum infiltrated and incubated at 37 C for 3 days, at which time the blue color reaction at the end of inoculated wounds was monitored with the naked eye or a dissecting microscope (4–12×).

Statistical analyses. Statistical analyses were conducted with the general linear models procedures of the Statistical Analyses Systems (SAS Institute, Cary, NC). Treatment means were compared with the Waller-Duncan K ratio t test ($P = 0.05$).

RESULTS

Tumor induction in the greenhouse. Table 2 shows the results of shoot inoculations with different strains of *A. vitis* and *A. tumefaciens* isolated from grapevine. In all combinations, *V. vinifera* 'Chasselas' was the most sensitive. Visible tumors developed on inoculated shoots of Chasselas within 4–6 wk. All strains induced large tumors on Chasselas, and the weights of tumors induced by different strains were not statistically different. In general, tumors appeared later on rootstocks than on *V. vinifera*. Small swellings could be seen after 6 wk, but measurable tumors were observed after 2–5 mo. The *V. riparia* clones (Gloire K and sauvage) were the most resistant. Only strain AB3, a strain with a small TA region and a limited host range, was able to induce measurable tumors on them. The other plant-bacterium combinations showed intermediate susceptibilities. The degree of pathogenicity varied for bacterial strains depending on the grape cultivar. No single strain was most pathogenic on all cultivars.

Multiplication of bacteria in plant tissue. Strain 2/3S5R of *A. vitis* multiplied in Gloire as well as in Chasselas (Fig. 1). In preliminary experiments, it was determined by sectioning the petiole tissue surrounding the inoculation site (2-mm cross sections) that in both genotypes, more than 99% of the bacteria remained localized in the 10-mm-long petiole tissue surrounding the wound. The results clearly show that the multiplication of bacteria in the resistant cultivar Gloire is not inhibited, and apparently the failure of tumor formation is not related to the restricted growth of bacteria in this genotype.

Root decay. Lesions were induced on young roots of all *Vitis* genotypes (Table 2) by *A. vitis* strains (AT-1, Tm4, AB3, 2/3, CG49, and Ag162). Biotype 1 strains (16/6 and 1/21) did not induce any root decay. The dark, sunken lesions typical of infection on *V. vinifera* cultivars were also observed on all other *Vitis* genotypes.

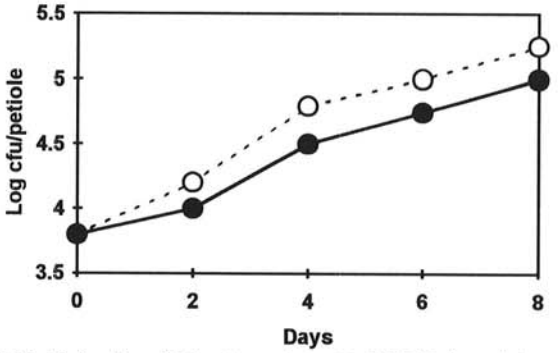

Fig. 1. Multiplication of *Agrobacterium vitis* 2/3S5R in petioles of *Vitis vinifera* 'Chasselas' (—●—) and *V. riparia* 'Gloire de Montpellier' (- - -○- - -). Petioles were wounded with a scalpel and inoculated with bacterial suspensions. Inoculated petioles were removed from plants at the indicated times and ground in distilled water. Bacterial populations were assessed by dilution plating. This experiment was performed four times. Data are not significantly different at $P = 0.05$.

TABLE 3. Opine contents of grapevine tumors induced by *Agrobacterium* strains

Vitis sp. Cultivar	*A. tumefaciens*[z] 16/6	*A. tumefaciens*[z] 1/21	*A. vitis*[z] AT-1	*A. vitis*[z] AB3	*A. vitis*[z] Tm4	*A. vitis*[z] 2/3	*A. vitis*[z] CG49	*A. vitis*[z] Ag162
V. riparia								
Gloire	–	–	–	–	–	–	–	–
Gloire K	–	–	–	–	–	–	–	–
selecta	nop	nop	nop	–	–	–	nop	–
sauvage	–	–	nop	–	–	–	nop	–
tomentosa	–	–	nop	oct	–	nop	nop	–
Martin	–	nop	nop	–	–	nop	–	–
V. riparia × *V. cordifolia*	nop	nop	nop	oct	–	nop	–	–
V. riparia × *V. rupestris*								
287C	–	–	–	–	–	–	nop	–
101-14Mgt	nop	–	nop	–	–	–	nop	–
V. berlandieri × *V. rupestris* Paulsen 775	nop	nop	nop	oct	oct	nop	nop	–
V. berlandieri × *V. riparia*								
Teleki 5BB	nop	nop	nop	oct	oct	nop	nop	oct
Teleki 8B	nop	nop	nop	oct	oct	nop	nop	oct
Teleki 5C	nop	nop	nop	oct	oct	nop	nop	oct
V. vinifera								
Chasselas	nop	nop	nop	oct	oct	nop	nop	oct

[z] – = Opine was not isolated; nop = nopaline; and oct = octopine. Isolated by paper electrophoresis.

TABLE 4. Induction of the *vir* gene and β-glucuronidase (GUS) activity of wounded tissues of *Vitis* spp.

Vitis sp. Cultivar	*vir* gene induction[y]	GUS activity[y,z]
V. riparia		
Gloire		
Shoots	++++	+ 8/10
Roots	++++	– 1/10
Gloire K	++++	nt
selecta	++++	nt
sauvage	++++	nt
tomentosa	++++	nt
Martin	++++	nt
V. riparia × *V. cordifolia*	++++	nt
V. riparia × *V. rupestris*		
287C	++++	nt
101-14Mgt	++++	nt
V. berlandieri × *V. rupestris* Paulsen 775	++++	nt
V. berlandieri × *V. riparia*		
Teleki 5BB	++++	nt
Teleki 8B	++++	nt
Teleki 5C	++++	nt
V. vinifera		
Chasselas		
Shoots	++++	++++ 10/10
Roots	++++	++++ 10/10

[y] + = Weak activity, ++++ = strong activity, and – = no activity.
[z] Positive/total. nt = Not tested.

Opine synthesis. Opines were not detected in Gloire (Table 3), although AB3 (a strain with a limited host range) induced some observable tumors from which isolations were attempted. *V. riparia* 'sauvage' (a wild American grape and a clone of *V. riparia*) produced detectable amounts of nopaline in tumors that were caused by AT-1 and CG49. In other cases, at least two attempts were made to recover opines from each inoculation site. If no opines were recovered, they may have been present below detectable levels.

Induction of the *vir* gene. Shoot and root segments were assayed for *vir*-inducing activity. In our assay, plant tissues were placed directly on an agar plate containing X-Gal with a lawn of the indicator bacterium. Blue coloration appeared at the wound sites of the shoots and along the roots after 24–48 h. Both shoot and root segments of all the *Vitis* spp. produced compounds that were able to induce *vir* gene activity (Table 4).

GUS assay. In order to determine whether T-DNA was transferred to plant cells, GUS activity was observed with strain CG49 (*A. vitis*) carrying the plasmid p35SGUSintron (23). Excised grape shoots and roots cultivated in vitro were cocultivated with strain CG49 pTiCG49, p35SGUSintron for 3 days at 25 C in the dark and stained with the GUS substrate X-Gluc. All 10 shoots and roots of Narancsizü were GUS positive 3 days after inoculation (Table 4). The blue indigogenic dye covered entire cut ends of shoots and roots. The GUS expression of Gloire varied considerably. Generally, wounds on Gloire were much less intensively stained than were wounds on Narancsizü. Two of 10 shoot segments and nine of 10 root segments of Gloire were not stained at all (Table 4). Observations of inoculated tissues with a microscope, however, showed that all visually weakly positive shoots of Gloire had some blue spots in the sections similar to those seen in Narancsizü. None of the controls showed any blue color.

DISCUSSION

Significant differences were found in resistance among different *Vitis* genotypes and rootstocks to the strains of *A. vitis* and *A. tumefaciens*. *V. riparia* 'Gloire' was the most resistant and *V. vinifera* 'Chasselas' the most susceptible, regardless of the strain of *Agrobacterium* used. *V. riparia* was also found to be among the genotypes most resistant to crown gall tested by Heil (5) and Stover (19). Teleki 5C, a widely used rootstock in both Hungary and the United States, was among the most susceptible genotypes in our experiments. In the field, it was observed that scions grafted on Teleki 5C were more susceptible to the disease than those grafted on 5BB (S. Süle *unpublished data*). Goodman et al (4) also found Teleki 5C to be highly susceptible to crown gall. Contrary to our result, Stover (19) found that Teleki 5C belonged to the least susceptible category. The discrepancy may be related to the confusion over the identity of grape rootstocks (19) or to differences in strains of *A. vitis* that were used. Similarly, Paulsen 775 was found to be immune in South Africa (3) and of intermediate susceptibility in our assays. The possible explanation for this difference may be related to the different pathogen strains used or to the use of different inoculation methods. The underlying causes of the differences are unknown, but resistance could result from several factors, including failure of *Agrobacterium* to attach to host cells and to multiply and survive at the wound sites, the lack of *vir* induction at the wound sites, failure of T-DNA to integrate into plant cells, or the inability of wounded plant cells to abnormally proliferate after infection. We have demonstrated that *V. riparia* 'Gloire' is resistant to most of the strains of *A. vitis* and *A. tumefaciens;* that *A. vitis* multiplied at the same rate in the resistant *V. riparia* as in the susceptible *V. vinifera;* that all *A. vitis* strains produced root decay on all *Vitis* genotypes; that all *Vitis* genotypes produced *vir*-inducing compounds; and that the T-DNA of *A. vitis* was transferred to

V. riparia cells, although at a lower frequency than to *V. vinifera* cells. Therefore, we hypothesize that the T-DNA is expressed only transiently. The stable incorporation of T-DNA genes may somehow be inhibited or not expressed at the level required for normal tumorigenesis. As a consequence, the infected plant cells do not form tumors as in the susceptible plant response. The resistance may be related to reduced efficiency in steps in T-DNA transfer, integration into host cells, expression of T-DNA genes, or plant response to the T-DNA–coded phytohormone production. In *Nicotiana glauca,* wild-type strains induced high levels of *gusA* expression early after infection, but the *gusA* expression appeared to be lost some time after infection in the infected leaf discs (11).

Our hypothesis is supported by our failure to detect opines in the infected *V. riparia* 'Gloire' tissues. However, this may have been caused by the insensitivity of our opine detection method or by some compounds that interfered with the visualization of opines after electrophoresis.

It was suggested that the *vir* induction is not only a prerequisite of T-DNA transfer, but also correlates with tumorigenicity (6). The hypothesis that in resistant genotypes, *vir* is not induced or is induced at lower levels was not supported by our experiments. Shoots and roots from all genotypes induced *vir* to the same extent. Thus, it can be concluded that *vir* induction was not the limiting factor in the tumorigenesis of the resistant cultivar Gloire.

Compounds produced at wound sites of some plant species, such as maize, may have an inhibitory effect on bacterial virulence. DIMBOA was found to inhibit both *vir* induction and the bacterial growth rate (18). The resistance of *V. riparia,* however, can not be related to the inhibition of bacterial growth, because in our assay there were no significant differences in the numbers of viable bacteria recovered from wound sites of *V. riparia* compared with those recovered from *V. vinifera. A. vitis* was able to multiply at the same rate in both *Vitis* genotypes. A similar result was found in *Pisum sativum* 'Sweet Snap.' In the wounds of this resistant cultivar, *A. tumefaciens* cells survived as well as those in the wounds of the susceptible cultivar Wando (16).

In conclusion, we compared the responses of different *Vitis* genotypes to different *A. vitis* and *A. tumefaciens* strains. In all combinations, *V. riparia* 'Gloire' was the most resistant. The incompatibility of this species with *Agrobacterium* strains does not appear to result from lack of bacterial multiplication or from lack of *vir* gene induction. Rather, the extent of stabile T-DNA integration or the inability of the transformed cells to proliferate after T-DNA transfer may be the basis for the apparent resistance exhibited.

LITERATURE CITED

1. Baribault, T. J., Skene, K. G. M., Cain, P. A., and Scott, N. S. 1990. Transgenic grapevines: Regeneration of shoots expressing β-glucuronidase. J. Exp. Bot. 41:1045-1049.
2. Brisset, M., Rodriguez-Palenzuela, P., Burr, T. J., and Collmer, A. 1991. Attachment, chemotaxis, and multiplication of *Agrobacterium tumefaciens* biovar 1 and biovar 3 on grapevine and pea. Appl. Environ. Microbiol. 57:3178-3182.
3. Ferreira, J. H. S., and van Zyl, F. G. H. 1986. Susceptibility of grape-vine rootstocks to strains of *Agrobacterium tumefaciens* biovar 3. S. Afr. J. Enol. Vitic. 7:101-104.
4. Goodman, R. N., Grimm, R., and Frank, M. 1993. The influence of grape rootstocks on the crown gall infection process and tumor development. Am. J. Enol. Vitic. 44:22-26.
5. Heil, M. 1993. Untersuchungen zur Resistenz von *Vitis* gegen *Agrobacterium tumefaciens.* Ph.D. diss. University of Hohenheim, Hohenheim, Germany.
6. Hood, E., Helmar, G., Fraley, R., and Chilton, M.-D. 1986. The hypervirulence of *Agrobacterium tumefaciens* A281 is encoded in a region of pTiBo542 outside of T-DNA. J. Bacteriol. 168:1292-1301.
7. Jefferson, R. 1987. Assaying chimeric genes in plants: The GUS gene fusion system. Plant Mol. Biol. Rep. 5:387-405.
8. Knauf, V. C., Panagopoulos, C. G., and Nester, E. W. 1982. Genetic factors controlling the host range of *Agrobacterium tumefaciens.* Phytopathology 72:1545-1549.
9. Mcguire, R. G., Rodriguez-Palenzuela, P., Collmer, A., and Burr, T. J. 1991. Polygalacturonase production by *Agrobacterium tumefaciens* biovar 3. Appl. Environ. Microbiol. 57:660-664.
10. Melchers, L. S., and Hooykaas, P. J. J. 1987. Virulence of *Agrobacterium.* Pages 167-220 in: Oxford Surveys of Plant Molecular and Cell Biology. B. J. Miflin, ed. Oxford University Press, Oxford.
11. Mozo, T., and Hooykaas, P. J. J. 1992. Factors affecting the rate of T-DNA transfer from *Agrobacterium tumefaciens* to *Nicotiana glauca* plant cells. Plant Mol. Biol. 19:1019-1030.
12. Murashige, T., and Skoog, G. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
13. Ophel, K., and Kerr, A. 1990. *Agrobacterium vitis*—New species for strains of *Agrobacterium* biovar 3 from grapevine. Int. J. Syst. Bacteriol. 40:236-241.
14. Paulus, F., Canaday, J., and Otten, L. 1991. Limited host range Ti plasmids: Recent origin from wide host range Ti plasmids and involvement of a novel IS element, IS*868.* Mol. Plant-Microbe Interact. 4:190-197.
15. Paulus, F., Huss, B., Bonnard, G., Ride, M., Szegedi, E., Tempe, J., Petit, A., and Otten, L. 1989. Molecular systematics of biotype III Ti plasmids of *Agrobacterium tumefaciens.* Mol. Plant-Microbe Interact. 2:64-74.
16. Robbs, S. L., Hawes, M. C., Lin, H.-J., Pueppke, S. G., and Smith, L. Y. 1991. Inheritance of resistance to crown gall in *Pisum sativum.* Plant Physiol. 95:52-57.
17. Rogers, S. G., Horsch, R. B., and Fraley, R. T. 1988. Gene transfer in plants: Production of transformed plants using Ti plasmid vectors. Pages 423-436 in: Methods for Plant Molecular Biology. A. Weissbach and H. Weissbach, eds. Academic Press, San Diego, CA.
18. Sahi, S. V., Chilton, M.-D., and Chilton, W. S. 1990. Corn metabolites affect growth and virulence of *Agrobacterium tumefaciens.* Proc. Natl. Acad. Sci. USA 87:3879-3883.
19. Stover, E. W. 1993. Resistance to crown gall in *Vitis:* Studies directed toward the identification of crown gall resistant rootstocks. Ph.D. diss. University of Maryland, College Park.
20. Süle, S. 1978. Biotypes of *Agrobacterium tumefaciens* in Hungary. J. Appl. Bacteriol. 44:207-213.
21. Szegedi, E., Czako, M., Otten, L., and Koncz, C. S. 1988. Opines in crown gall tumors induced by biotype 3 isolates of *Agrobacterium tumefaciens.* Physiol. Mol. Plant Pathol. 32:237-247.
22. Szegedi, E., Korbuly, J., and Koleda, I. 1984. Crown gall resistance in East-Asian *Vitis* species and their *V. vinifera* hybrids. Vitis 23:21-26.
23. Vancanneyt, G., Schmidt, R., O'Connor-Sanchez, A., Willmitzer, L., and Rocha-Sosa, M. 1990. Construction of an intron-containing marker gene: Splicing of the intron in transgenic plants and its use in monitoring early events in *Agrobacterium*-mediated plant transformation. Mol. Gen. Genet. 220:245-250.
24. Winans, S. C., Kerstetter, R. A., and Nester, E. W. 1988. Transcriptional regulation of the *virA* and *virG* genes of *Agrobacterium tumefaciens.* J. Bacteriol. 170:4047-4054.