Biological Control of Grape Crown Gall with Non-tumorigenic *Agrobacterium vitis* Strain F2/5

T. J. BURR[1]* and C. L. REID[2]

A non-tumorigenic strain (F2/5) of *Agrobacterium vitis* produces an agrocin that inhibited *in vitro* growth of 21 of 25 *A. vitis*, two of 10 *A. tumefaciens* biovar 1, and none of nine biovar 2 strains. It inhibited both tumorigenic and non-tumorigenic strains. When applied to wounds on potted woody grape trunks (*Vitis vinifera* L. cvs. Chardonnay and Riesling) in the greenhouse, the gall sizes were significantly reduced for seven of 10 *A. vitis*, one of two *A. tumefaciens* biovar 1 and one of one biovar 2 strains. The numbers of inoculation sites at which galls developed was reduced for all but one *A. vitis* strain. There is a good, but not perfect, correlation between *in vitro* sensitivity to the agrocin and control *in vivo*. Co-inoculation of F2/5 with pathogen was as effective or more effective in most cases than pre-inoculation of F2/5. When pathogen was inoculated prior to F2/5, the level of control was greatly reduced. Control was most effective when equal concentrations of F2/5 and pathogen were inoculated and declined for ratios of 1:10 and 1:100 (F2/5 to pathogen). F2/5 contains three plasmids, none of which hybridize with a probe, pTHE17, consisting of the T-DNA from *A. tumefaciens* strain C58.

KEY WORDS: *Agrobacterium vitis*, *A. tumefaciens*, [*A. tumefaciens* biovar 3], crown gall, biological control.

Agrobacterium vitis (16) formerly designated as *A. tumefaciens* biovar 3 or biotype 3, is the most predominant species causing crown gall disease on grape (3). The bacterium survives systemically in grape and is frequently disseminated in propagation material (4). Recently, methods have been tested for developing pathogen-free vines using shoot tip culture (6) and heat therapy (7). However, once clean vines are obtained, it will be necessary to protect them from reinfection from *A. vitis* inoculum that may persist in decaying grape debris in vineyard soils (1,3,5).

Biological control of crown gall has been highly successful on several crops using *A. radiobacter* strain K-84 (12); however, *A. vitis* is not controlled by this strain (3). Therefore, several laboratories have attempted to identify biological controls that are effective against *A. vitis* (13,17,19,20). Xiaoying and Wangnian (21) isolated a non-tumorigenic strain of biovar 1 (HLB-2) that inhibited growth of several *A. vitis* strains and suppressed development of the disease on grape in the greenhouse. Staphorst evaluated 16 strains including strain F2/5 which was effective against some *A. vitis* strains in laboratory and in greenhouse tests (18). The purpose of this research was to test F2/5 against several strains of *Agrobacterium* from different geographical regions. F2/5 was evaluated for *in vitro* activity and for control on grape in the greenhouse. Ratios of pathogen to F2/5 were tested as well as timing of application of F2/5 to in relation to inoculation with the pathogen. Plasmid content of F2/5 was compared to that of K-84 and HLB-2 and Southern analysis was done to determine if native plasmids of F2/5 hybridize with a DNA probe consisting of T-DNA from *A. tumefaciens* strain C58 (8).

Materials and Methods

Bacterial strains: Strains used are listed in Table 1. All strains were stored at -80°C in cryogenic storage medium (1.2 g nutrient broth, 22.5 g glycerol, 85 mL distilled water). Inocula for experiments were grown on potato dextrose agar (PDA, Difco) or MG medium (10). Strain F2/5, previously referred to as F2 (17), was kindly provided by Dr. J. L. Staphorst (Plant Protection Research Institute, Private Bag X14, Pretoria 0001, Rep. of South Africa) and obtained by APHIS permit No. 1 PPQ 584.

***In vitro* assay:** Evidence for the production of an agrocin was obtained by placing a 10 µL drop of a suspension of strain F2/5 containing about 10^8 colony-forming units (CFU) (optical density of 0.1 at 600 nm determined with spectrophotometer) was applied to the center surface of a 9-cm petri plate containing 20 mL of MG medium. Plates were incubated for 48 hours at 28°C, at which time bacterial cells were killed by chloroform vapor, and bacterial growth was scraped from the plates. Subsequently, the surface of the plates were sprayed until wetted with suspensions (about 10^8 CFU/mL) of bacteria to be tested for sensitivity. Forty-four *Agrobacterium* strains were tested for agrocin sensitivity including *A. tumefaciens* biovar 1 (5 tumorigenic, 6 non-tumorigenic), biovar 2 (5 tumorigenic and 5 non-tumorigenic), and *A. vitis* (17 tumorigenic and 6 non-tumorigenic). Plates were incubated at 28°C, and the sizes of inhibition zones were recorded after 48 hours.

[1]Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

*Author to whom correspondence should be addressed.

This research was partially funded by the New York State Wine and Grape Foundation and UST Inc.

Manuscript submitted for publication 4 November 1993.

Table 1. Sensitivity of *Agrobacterium vitis* and *A. tumefaciens* biovars 1 and 2 to agrocin produced by strain F2/5.

Strain	Source[a]	Biovar or species	Tumor[b]	Sens. to[c] agrocin F2/5
CG628	Burr, grape, NY	1	+	-
CG632	Burr, grape, NY	1	+	-
CG640	Burr, grape, NY	1	+	-
CG648	Burr, grape, NY	1	+	-
CG90	Burr, grape, NY	1	-	-
CG91	Burr, grape, NY	1	-	-
CG210	Burr, grape, NY	1	-	+
CG219	Burr, grape, NY	1	-	-
C58	Dickey, cherry, NY	1	-	++
HLB2	Xiaoying, hop, China	1	-	-
CG907	Burr, raspberry NY	2	+	-
A-4	Ark, rose, CA	2	+	-
K-47	Kerr, Australia	2	+	-
R-3	Dickey,rose, NY	2	+	-
CG978	Burr, pussy willow, NY	2	+	-
CG414	Burr, soil, NY	2	-	-
CG423	Burr, soil, NY	2	-	-
CG438	Burr, grape root, WA	2	-	-
K84	A. Kerr, peach, Australia	2	-	-
CG47	Burr, NY	Av[d]	+	++
CG49	Burr, NY	Av	+	+++
CG56	Burr, MI	Av	+	+
CG60	Burr, NY	Av	+	++++
CG78	Burr, NY	Av	+	++
CG98	Burr, VA	Av	+	++++
CG102	Burr, VA	Av	+	++++
CG106	Burr, MS	Av	+	+++
CG108	Burr, NM	Av	+	++++
CG660	Burr, NY	Av	+	-
CG693	Burr, NY	Av	+	+
CG696	Burr, NY	Av	+	++
Ag57-81	Panagopoulos, Crete	Av	+	++++
AA 25	Ercolani, Afghanistan	Av	+	+++
1860 (3)	Bazzi, Italy	Av	+	+
NW-161	Bien, Germany	Av	+	++++
K306	Ophel, Australia	Av	+	++
CG472	Burr, WA	Av	-	++
CG481	Burr, NY	Av	-	-
CG482	Burr, WA	Av	-	++++
CG483	Burr, WA	Av	-	++++
CG487	Burr, WA	Av	-	-
CG488	Burr, WA	Av	-	++++
CG669	Burr, grape, NY	?[e]	-	-
CG670	Burr, grape, NY	?	-	+

[a]Author, plant from which isolated, and state or country.

[b]Tumorigenicity as determined on sunflower, kalanchoe, tomato, and/or grape.

[c]Relative size of inhibition zone (mm): -= no inhibition, $0 < + \leq 1$, $1 < ++ \leq 2$, $2 < +++ \leq 3$, $3 < ++++$.

[d]Av = *A. vitis*. All strains were isolated from grape.

Assay on grape: Rooted grape, *Vitis vinifera L.* cuttings (about one-month-old cvs. Chardonnay and Riesling), were used. They had been stored prior to planting as cuttings of dormant canes with three nodes . Inoculations with bacteria were made by applying 75 µL of bacterial cell suspensions in holes that were bored in the living woody stems of the plants with an electric drill. The drill bit diameter was about 6 mm and holes were drilled to the depth of the pith. Three or four inoculations were made to each plant and inoculation sites were wrapped with parafilm. The numbers of inoculation sites at which galls developed and gall size (mm^2 of gall surface area) were recorded 8 weeks after inoculation. Sterile distilled water was applied as a negative control. Data were analyzed using the Waller-Duncan K-ratio test.

Biological control activity of F2/5 against different strains of *A. tumefaciens* was tested. Wound sites were first inoculated with 75 µL of a suspension (about 10^8 CFU/mL) of F2/5 or sterile distilled water. About 90 minutes later, they were inoculated with the same volume and concentration of *A. vitis* or *A. tumefaciens* strains. To test the effect of applying the pathogen and F2/5 at the same time, a suspensions containing equal concentrations of F2/5 and pathogenic strains (10^8 CFU/mL for each) were inoculated to wound sites.

We also tested the effects of applying F2/5 at different times in relation to inoculation with the pathogen. Suspensions of F2/5 and *A. vitis* strain K306, of equal concentration (about 10^8 CFU/mL) were used. In one experiment, F2/5 or sterile distilled water were applied about 10 minutes, five hours, and 16 hours prior to inoculation with K306. In another experiment, strain K306 or sterile distilled water were applied to wound sites and F2/5 was applied about 10 minutes, three hours, six hours, or 18 hours later. All experiments were repeated at least once.

Biological control as affected by concentration of F2/5 in relation to concentration of the pathogen was tested using ratios of 1:1, 1:10, and 1:100 of F2/5 to pathogen. In these experiments, F2/5 was applied at a concentration of about 10^8 CFU/mL 90 minutes before inoculation with pathogen. *A. vitis* strains included CG106 and K306. Pathogens and F2/5 were also applied alone to plants as described above. Suspensions of pathogens and F2/5 were serially diluted and plated on PDA medium to verify populations and

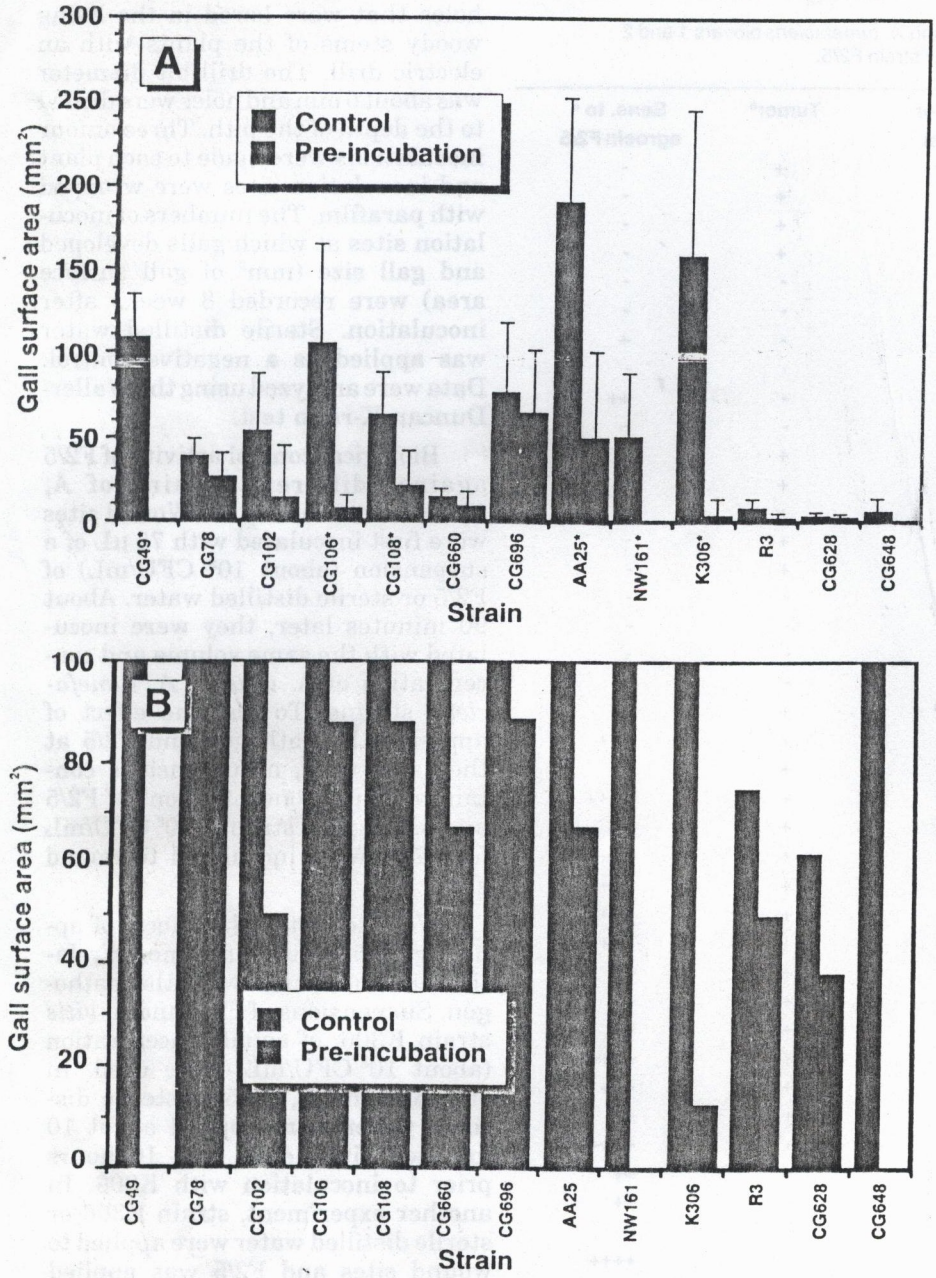

Fig. 1. (A) Effect of pre-inoculation of F2/5 on gall size. (B) The percentage of inoculation sites at which galls developed.

ratios. All experiments were repeated at least once.

Agrocin effect in mixed inoculations: The effect that secreted agrocin in F2/5 cultures may have on survival of cells of the pathogen that were co-mixed with F2/5 prior to inoculation was determined. Cultures of F2/5 and strains K306, CG49, and CG51 (spontaneous rifampicin mutant of CG49) were grown on PDA or PDA plus rifampicin (50 µg/mL) and then suspended in distilled water to a concentration of about 10^8 CFU/mL. F2/5 and single pathogenic strains were mixed at equal concentrations in distilled water and incubated at 25°C. Populations of pathogen and F2/5 were measured immediately after mixing and after one and four hours by plating on PDA (or PDA plus rif) and on RS medium (F2/5 only produces small colonies on RS that are distinct from strains CG49 and K306). Plates were incubated 48 hours (PDA) or six days (RS) at 28°C, and colonies were counted. The experiment was repeated once.

Southern analysis of F2/5 plasmids: Plasmid DNA was isolated from *Agrobacterium* strains K-84 , HLB-2 and F2/5, and CG56 using the method of Slota and Farrand (17). Undigested plasmids were electrophoresed in 0.7% agarose in TBE (14) at 5 V/cm. DNA was stained with ethidium bromide and visualized. DNA was then Southern transferred to GeneScreen Plus-Hybridization Transfer Membrane (Dupont, NEN Research Products) by alkaline transfer. Hybridizations were done with T-DNA probe, pTHE17 (8) which was labeled by random primed incorporation of digoxigenin-labeled deoxyuridine-triphosphate using a non-radioactive DNA labeling kit (Genius, Boehringer Mannheim, Indianapolis, IN).

Membranes were pre-hybridized at 68°C for 135 minutes and hybridized at 68°C for 18 hours in 0.03 mL hybridization solution/cm² membrane containing 25 ng/mL of labeled probe pTHE17. Following post-hybridization, rinses, blocking, and incubation with antibody-conjugate solution and subsequent rinses, the membrane was placed within a hybridization bag that was open on two sides and saturated with a sufficient quantity of LumiPhos 530 (Boehringer Mannheim, for chemiluminescent detection of alkaline phosphatase) to saturate the membrane. It was then incubated in the dark (wrapped in aluminum foil) for one minute. The excess LumiPhos 530 was then drained from the hybridization bag, the bag was sealed and wrapped in foil and incubated at 37°C for 30 minutes. The membrane was subsequently placed in a film cassette, exposed to X-ray film (Kodak XAR) for eight minutes, and then developed.

Results

***In vitro* assay:** F2/5 produces an agrocin that is primarily inhibitory to *A. vitis* strains (Table 1). However, two *A. tumefaciens* biovar 1 strains (CG210 and C58) were sensitive and three *A. vitis* strains (CG660, CG481, and CG487) were not. Sensitivity to agrocin was not correlated with tumorigenicity, since some

C3

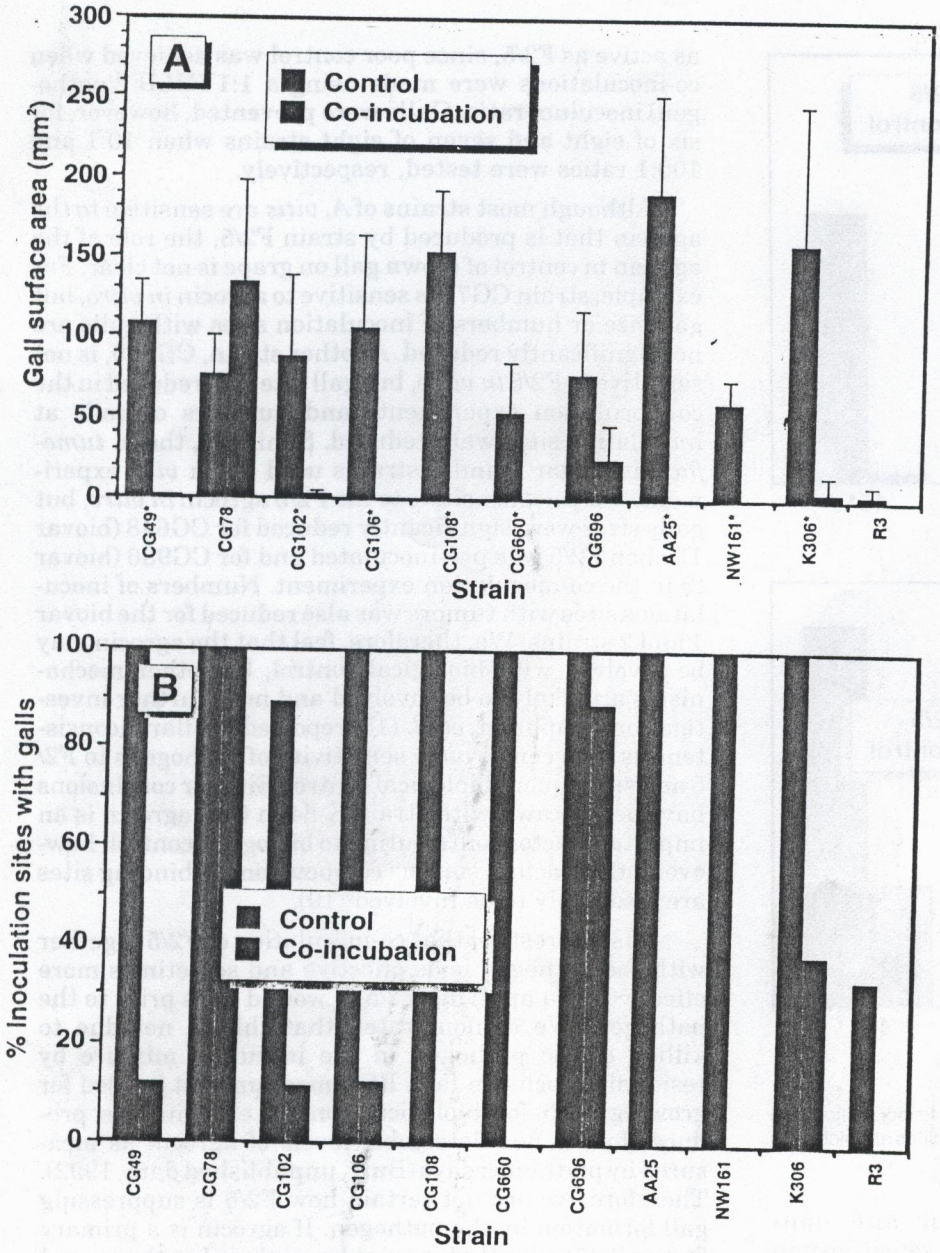

Fig. 2. (A) Effect of coinoculation of F2/5 and *Agrobacterium* strains on gall sizes. (B) The percentage of inoculation sites at which galls developed.

tumorigenic and non-tumorigenic strains of *A. vitis* were highly sensitive as determined by relative inhibition zone sizes that were produced on bioassay plates.

***In vivo* activity:** F2/5 significantly reduced gall size caused by seven of 10 *A. vitis*, 1 of 2 *A. tumefaciens* biovar 1 and 1 biovar 2 strains when applied prior to inoculation with the pathogen (Fig. 1A). It reduced the numbers of galls on plants inoculated with all strains except one, *A. vitis* (CG78) (Fig. 1B). When applied at the same time as the pathogen (co-inoculated), it significantly reduced gall sized and the number of galls produced for eight of nine *A. vitis* strains and one *A. tumefaciens* biovar 2 (Fig. 2A and 2B). Again F2/5 did not affect gall formation by CG78. In no case did F2/5 induce galls.

F2/5 was most effective when applied at the same concentration as the pathogen (Fig. 3). At a ratio of 1:10, (F2/5 to pathogen) galls sizes for strain CG106, but not for K306, were smaller than the control. At 1:100, gall sizes for both strains were not different from the control. The number of galls induced by CG106 was not greatly reduced when F2/5 was preinoculated at the 1:1 ratio, but was significantly reduced when CG106 was co-inoculated with F2/5.

F2/5 was most effective in reducing tumor size and number of inoculation sites with galls when applied prior to or at the same time as inoculation of the pathogen (Fig. 4). When the pathogen was applied prior to F2/5, gall size and numbers of inoculation sites with tumors were not different from plants that were only inoculated with the pathogen.

Agrocin effect in mixed inoculum: There was no detectable effect of residual agrocin from F2/5 on *A. vitis* survival when mixtures of the strains were made in water. The populations of F2/5 in the inoculum mixtures equaled 4.3×10^7 CFU/mL, whereas CG49, CG51, and K306 were 4.9×10^7, 1.5×10^7, and 2.5×10^7, respectively. After four hours in the mixture with F2/5. populations of CG49, CG51, and K306 were 7.7×10^7, 3.7×10^7, and 4.3×10^7, respectively.

Plasmid profiles and Southern analysis: Plasmid profiles of K-84, HLB-2, and CG56 were identical to those previously reported (8). Strain F2/5 contains three plasmids (Fig. 5A) one being smaller than typical plasmids associated with tumorigenic *Agrobacterium* (Ti-plasmids). Whereas a single plasmid from K-84, HLB-2, and CG56 hybridized with pTHE17, there was no hybridization with F2/5 (Fig. 5B).

Discussion

Several aspects that are important to the development of commercial usage of F2/5 have been determined. F2/5 is an *A. vitis* strain that reacts with a species-specific monoclonal antibody (2) and produces a secreted polygalacturonase (15) (Burr, unpublished data, 1992). It is non-tumorigenic, was originally isolated from grape (17), and therefore like other *A. vitis* strains, is likely to survive well epiphytically and endophytically on grape (1,3,4,5). It also produces an agrocin that is

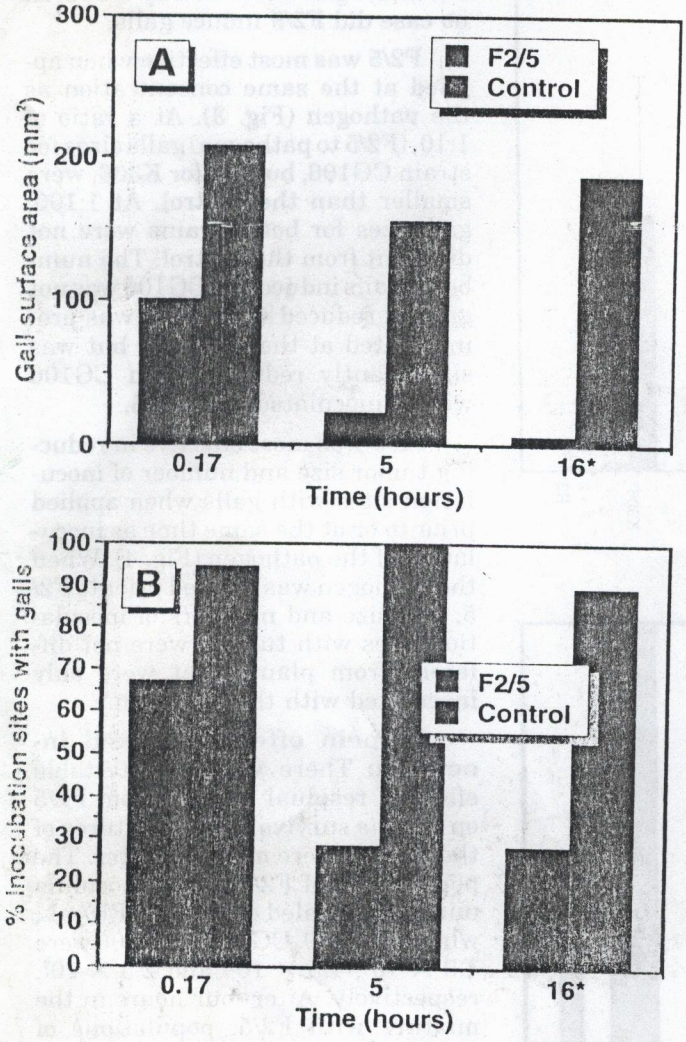

Fig. 3. (A) Effect of time of application of F2/5 in relation to inoculation with pathogen on gall size. (B) The percentage of inoculation sites at which galls develop.

primarily active against tumorigenic and non-tumorigenic *A. vitis* strains. In contrast, sensitivity to the agrocin produced by strain K-84 is encoded by determinants on certain Ti plasmids (tumorigenic strains), and *A. vitis* strains are not sensitive (3). We have demonstrated that F2/5 is active against several *A. vitis* and *A. tumefaciens* strains and that the effectiveness of control will depend on applying F2/5 prior to infection by the pathogen and at concentrations equal to or greater than that of the pathogen. It will be essential now to conduct field experiments by treating *A. vitis*-free vines prior to planting in pathogen-infested soils.

Other potential biological controls for grape crown gall have been reported from laboratories in South Africa (19) and China (12,20). One strain from China, HLB-2, was recently evaluated against a group of *A. vitis* strains from the U.S. (9). HLB-2 is a non-tumorigenic *A. tumefaciens* biovar 1 strain that produces an agrocin that is active against several strains of *Agrobacterium*. Although it reduces gall formation *in vivo*, it may not be as active as F2/5, since poor control was achieved when co-inoculations were made using a 1:1 (HLB-2:pathogen) inoculum ratio. Galls were prevented, however, for six of eight and seven of eight strains when 10:1 and 100:1 ratios were tested, respectively.

Although most strains of *A. vitis* are sensitive to the agrocin that is produced by strain F2/5, the role of the agrocin in control of crown gall on grape is not clear. For example, strain CG78 is sensitive to agrocin *in vitro*, but gall size or numbers of inoculation sites with galls are not significantly reduced. Another strain, CG660, is not sensitive to F2/5 *in vitro*, but gall size was reduced in the co-inoculation experiments and numbers of galls at inoculation sites were reduced. Similarly, the *A. tumefaciens* biovar 1 and 2 strains used for *in vivo* experiments were not sensitive to the F2/5 agrocin *in vitro*, but galls sizes were significantly reduced for CG648 (biovar 1) when F2/5 was pre-inoculated and for CG936 (biovar 2) in the co-inoculation experiment. Numbers of inoculations sites with tumors was also reduced for the biovar 1 and 2 strains. We, therefore, feel that the agrocin may be involved with biological control, but other mechanisms are likely to be involved and need further investigation. Staphorst, *et al.* (18) reported similar inconsistencies between *in vitro* sensitivity of pathogens to F2/5 and subsequent biological control. Similar conclusions have been drawn with strain K-84 in that agrocin is an important factor contributing to biological control; however, other factors such as competition for binding sites are also likely to be involved (10).

It is interesting that co-inoculation of F2/5 together with the pathogen is as effective and sometimes more effective than applying F2/5 to wound sites prior to the pathogen. We demonstrated that this is not due to killing of the pathogen in the inoculum mixture by residual agrocin. In fact, PDA medium that is used for growing F2/5 for biological control experiments produces low to non-detectable levels of agrocin as measured by plate bioassays (Burr, unpublished data, 1992). Therefore, we are not certain how F2/5 is suppressing gall formation by the pathogen. If agrocin is a primary factor, it appears that it must be produced at the wound site on the plant. It is also possible that F2/5 is competes for binding sites or affects tumorigenisis in some other way.

The genetic determinants for agrocin production by F2/5 have not been identified. The mechanism of agrocin F2/5 sensitivity is apparently different from that of K-84 (agrocin 84) which is Ti plasmid dependent, since non-tumorigenic strains of biovar 3 may be sensitive. Strain F2/5 carries three plasmids, two of which are comparable in size to the Ti plasmid (about 200 Kb) and none of which hybridize with T-DNA probe, pTHE17. In contrast, a 200 Kb plasmid from K-84, known to encode nopaline catabolism, shares some homology with pTHE17 (8). This result supports greenhouse pathogenicity tests that demonstrate F2/5 is non-tumorigenic.

Conclusions

We have demonstrated that *A. vitis* strain F2/5 has

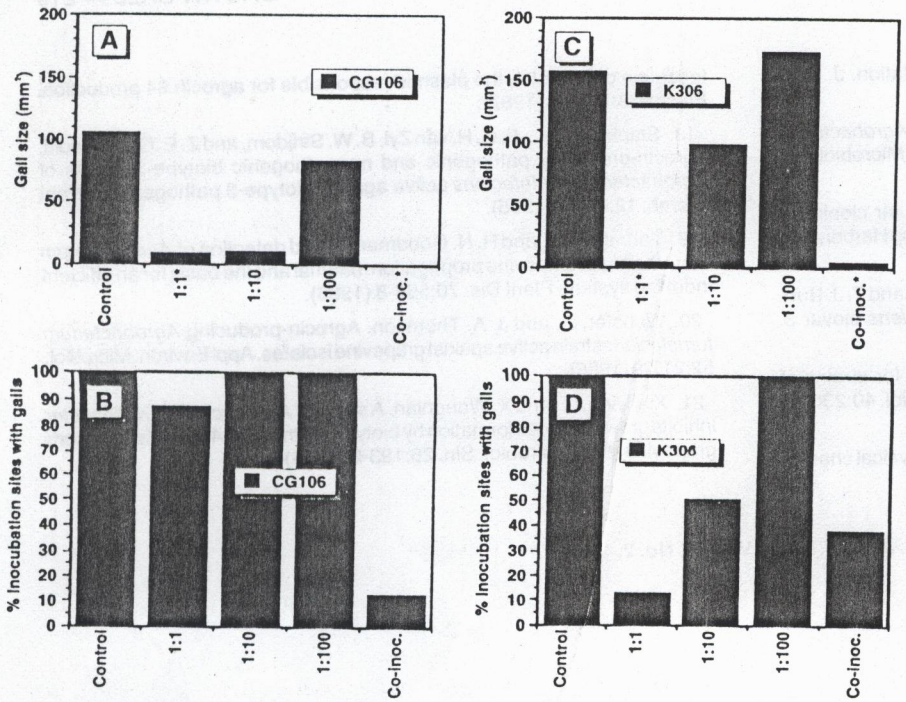

Fig. 4. (**A** and **C**) Effect of ratio of F2/5 to pathogen on galls size. (**B** and **D**) The percentage of inoculation sites at which galls develop.

potential as a biological control of grape crown gall. Because *A. vitis* is host-specific to grape, it is likely that F2/5 will survive and compete well on grape. Further research on the competitiveness of F2/5 in relation to tumorigenic *A. vitis* strains is necessary. F2/5 is clearly non-tumorigenic because of its failure to induce galls on plants in the greenhouse and to hybridize with a T-DNA probe. It will be essential to apply F2/5 to grapevines prior to infection and at a concentration equal to or greater than that of the pathogen. We propose that disease-free vines will be inoculated with F2/5 prior to planting in the vineyard. It will now be important to conduct field experiments with F2/5 by treating plants and growing them in *A. vitis*-infested soils. It would be best to conduct such trials in cold climates, where freeze injury predisposes plants to crown gall infection.

Literature Cited

1. Bishop, A. L., B. H. Katz, and T. J. Burr. Infection of grapevines by soilborne *Agrobacterium tumefaciens* biovar 3, and population dynamics in host and nonhost rhizospheres. Phytopathology 78: 945-8 (1988).

2. Bishop, A. L., V. L. Mittak, B. H Katz, and T. J Burr. A Monoclonal antibody specific to *Agrobacterium tumefaciens* biovar 3 and its utilization for indexing grapevine propagation material. Phytopathology 79:995-8 (1989).

3. Burr, T. J., and B. H Katz. Isolation of *Agrobacterium tumefaciens* biovar 3 from grapevine galls and sap and vineyard soil. Phytopathology 73:163-5 (1983).

4. Burr, T. J., and B. H. Katz. Grapevine cuttings as potential sites of survival and means of dissemination of *Agrobacterium tumefaciens*. Plant Dis. 68:976-8 (1984).

5. Burr, T. J., B. H. Katz, and A. L. Bishop. Populations of *Agrobacterium* in vineyard and nonvineyard soils and grape roots in vineyards and nurseries. Plant Dis. 71:617-20 (1987).

6. Burr, T. J., B. H. Katz, A. L. Bishop, C. A. Meyers, and V. L. Mittak. Effect of shoot age and tip culture propagation on grapes on systemic infestation by *Agrobacterium tumefaciens* biovar 3. Am. J. Enol. Vitic. 39:67-70. (1988).

7. Burr, T. J., K. Ophel, and A. Kerr. Effect of hot water treatment on systemic *Agrobacterium tumefaciens* biovar 3 in dormant grape cuttings. Plant Dis. 73:242-5 (1989).

8. Burr, T. J., J. L. Norelli, B. H. Katz, and A. L. Bishop. Use of Ti-plasmid DNA probes for determining tumorigenicity of *Agrobacterium* strains. Appl. Environ. Microbiol. 56:1782-5 (1990).

9. Cheng, Zong-Ming. Strategies for Genetic Engineering of Grapevines for Crown Gall Disease Resistance and Attempted Transformation via *Agrobacterium* Ti Plasmid Vectors and Biolistic Process. 193 pp. Cornell University (1991).

10. Cooksey, D. A., and L. W. Moore. Biological control of crown gall with an agrocin mutant of *Agrobacterium radiobacter*. Phytopathology 72:919-21 (1982).

11. Goodman, R. N., D. Butrov, and F. Tarbah. The occurrence of *Agrobacterium tumefaciens* in grapevine-propagating material and a simplified indexing system. Am. J. Enol. Vitic. 38:189-93 (1987).

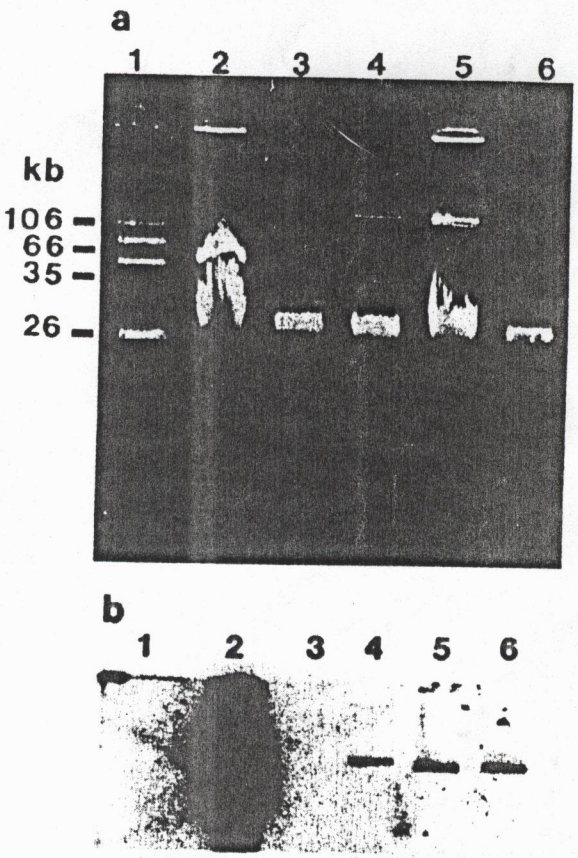

Fig. 5. (**A**) Plasmid profile. (**B**) Corresponding Southern analysis (hybidization with probe pTHE17) of strains F2/5 (lane 3), K84 (lane 4). HLB-2 (lane 5), and tumorigenic *A. vitis* strain CG56 (lane 6). Lane 1 is *Erwinia stewartii* strain SW2 (used as size marker, obtained from D. Coplin, Ohio State University) and lane 2 is *E. coli* strain DH-1 containing pTHE.

12. Kerr, A. Biological control of crown gall: seed inoculation. J. Appl. Bacteriol. 35:493-7 (1972).

13. Liang, Y., Y. Di, J. Zhao, and D. Ma. A biotype 3 strain of *Agrobacterium radiobacter* inhibits crown gall formation on grapevine. Acta Microbiol. Sin. 30:165-71 (1990).

14. Maniatis, T., E. F. Fritsch, and J. Sambrook. Molecular cloning: a laboratory manual. Cold Spring harbor Laboratory, Cold Spring Harbor, N.Y. (1982).

15. McGuire, R. G., P. Rodriguez-Palenzuela, A. Collmer, and T. J. Burr. Polygalacturonase production by *Agrobacterium tumefaciens* biovar 3. App. Environ. Micro. 57:660-4 (1991).

16. Ophel, K., and A. Kerr. *Agrobacterium vitis* sp. nov. for strains of *Agrobacterium* biovar 3 from grapevines. Int. J. Syst. Bacteriol. 40:236-41 (1990).

17. Slota, J. E., and S. K. Farrand. Genetic isolation and physical characterization of pAGK84, the plasmid responsible for agrocin 84 production. Plasmid 8:175-86 (1982).

18. Staphorst, J. L., F. G. H. van Zyl, B. W. Strijdom, and Z. E. Groenewold. Agrocin-producing pathogenic and nonpathogenic biotype-3 strains of *Agrobacterium tumefaciens* active against biotype-3 pathogens. Current Microb. 12:45-52 (1985).

19. Tarbah, F. A., and R. N. Goodman. Rapid detection of *Agrobacterum tumefaciens* in grapevine propagation material and the basis for an efficient indexing system. Plant Dis. 70:566-8 (1986).

20. Webster, J., and J. A. Thomson. Agrocin-producing *Agrobacterium tumefaciens* strain active against grapevine isolates. Appl Environ. Microbiol. 52:217-9 (1986).

21. Xiaoying, C., and X. Wangnian. A strain of *Agrobacterium radiobacter* inhibits growth and gall formation by biotype III strains of *A. tumefaciens* from grapevine. Acta Microbiol. Sin. 26:193-9 (1986).