

Crown Gall of Pecan: A Survey of *Agrobacterium* Strains and Potential for Biological Control in Georgia

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ABSTRACT

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Crown gall was found in numerous pecan orchards in Georgia. In some instances, 60% of the trees were diseased. Galled trees were less vigorous than uninfected trees and were often stunted. Among the pathogenic *Agrobacterium* strains isolated from 18 galled trees in six counties, biovar 1 strains predominated and most were sensitive to agrocin 84 in vitro. A representative biovar 1 strain from pecan was inhibited from infecting tomato seedlings by *A. radiobacter* strain K84. We would expect that biological control of crown gall in Georgia pecan orchards with the strain K84 would be successful. Additional antagonists for biocontrol of crown gall were isolated from potentially suppressive soil in a young peach orchard.

Pecan (*Carya illinoensis* (Wagenh.) C. Koch) sales in Georgia average more than \$50 million annually, and Georgia currently accounts for about 50% of the pecan production in the United States. This production comes from about 2 million bearing trees, with the highest concentration of trees in south central Georgia (8), where this study was done.

Rand (15) first reported crown gall on pecan trees grown in a nursery in Mississippi and in northern Florida. Today, crown gall is a major concern to many pecan growers whose trees are afflicted by large and numerous tumors at the base of the trunk and in the root system. This paper reports the incidence of crown gall in the pecan-growing counties of Georgia, the predominant biovar of *Agrobacterium tumefaciens*

isolated from infected trees, and the sensitivity of these strains to agrocin 84 produced in vitro by *A. radiobacter* K84. Such characterization is important in order to determine whether crown gall on pecan could be controlled with the bacteriocinogenic *A. radiobacter* strain K84 (14).

MATERIALS AND METHODS

Agrobacterium spp. were isolated from gall samples obtained mostly from trees of 40- to 60-yr-old pecan orchards. Incidence of diseased trees in these orchards ranged from 25 to 60%. Galled trees appeared less vigorous than ungalled trees and were often stunted. The trees were produced in Florida and Mississippi nurseries on unspecified pecan root stocks grafted with either Moneymaker, Schley, Stuart, or Teshe scions.

Galls from 18 trees were collected from orchards in Mitchell, Dougherty, Calhoun, Tift, and Baker counties. One gall sample from Jefferson County came from young pecan trees grown from nursery stock that were dipped 3 yr previously in the bacteriocinogenic *A. radiobacter* K84 before planting. These

treated trees were planted in soil where old pecan trees had been killed before replanting, reportedly from crown gall. To isolate for *A. tumefaciens*, the gall exteriors were washed thoroughly and 1 g of living tissue was ground with a mortar and pestle for 2 min in 10 ml of sterile distilled water. The suspension was left standing for 30 min and 0.1 ml of selected 10-fold serial dilutions was spread onto the selective media of Schroth et al (17), New and Kerr (13), and DIM (C. I. Kado and M. G. Heskett, unpublished) as described (12). The inoculated plates were incubated for 1 wk at 28 C. Fifteen colonies (five from each medium) resembling *Agrobacterium* were selected at random from each gall sample. After purification, the identity of each suspected strain of *Agrobacterium* was determined by the appropriate biovar 1 and 2 diagnostic tests (12).

Sensitivity of the characterized strains to agrocin 84 was tested on mannitol-glutamate agar plates (3) following Stonier's method (18) as modified by Cooksey and Moore (3). A 10- μ l sample of 10^7 colony-forming units (cfu) per milliliter suspension of *A. radiobacter* K84 was spotted in the center of each agar plate. After 2 days of incubation at 28 C, a 10^8 cfu/ml suspension of the strain to be tested was sprayed over the plates. Growth inhibition of the test strain was recorded after three additional days of incubation.

Pathogenicity tests were performed on stems of 4-wk-old seedlings of *Datura stramonium* and tomato (*Lycopersicon esculentum* 'Tampa') (2). Tumor formation was recorded 2 mo after inoculation. Biocontrol by K84 of two representative *A. tumefaciens* strains from pecan was tested on wounded tomato stems. One

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strain was a biovar 2 agrocin-resistant strain GA003 and the other was a biovar 1 agrocin-sensitive strain GA1010. *A. tumefaciens* strains B6 (R. Baker, Colorado State University) (biovar 1 and agrocin-resistant) and K24 (A. Kerr, University of Adelaide, South Australia) (biovar 2 and agrocin-sensitive) were included as reference strains. For each pathogenic strain, stems of 10 wounded tomato seedlings were each wounded longitudinally (slit 4–5 mm long) with a sterile scalpel blade and a 10- μ l suspension of K84 (10^8 cfu/ml) was applied to each wound with a micropipet. After 24 hr, these inoculated wounds were challenged with a 10- μ l suspension (10^7 cfu/ml) of a pathogenic strain.

Because all pathogenic strains are not subject to biological control by K84 (1,10), a search was made for other bacterial antagonists that would inhibit strains resistant to agrocin 84. Potential antagonists were isolated from the rhizospheres of healthy and galled peach and pecan trees in Brooks, Thomas, and Tift counties. Soil surrounding the roots was suspended in sterile distilled water, diluted serially, and 0.1 ml of the selected dilution was spread over duplicate plates of mannitol-glutamate agar medium. After two days of incubation at 28 C, plates with separated colonies were oversprayed with 10^8 cfu/ml suspensions of the agrocin-sensitive strain K24 or the agrocin-resistant strain B6. The plates were incubated for an additional 3–5 days at 28 C, and colonies producing compounds inhibitory to growth of both K24 and B6 were recovered, purified, and tested as described for K84.

RESULTS AND DISCUSSION

Nearly equal numbers of biovar 1 and biovar 2 strains were isolated from all the Georgia samples; however, there was a variability in the selectivity of the media. Of 81 *Agrobacterium* colonies selected from the three different media, 39 were biovar 1 strains and most of these were isolated on Schroth et al and DIM media (Table 1). In addition, three of four strains isolated earlier (N. W. Schaad, unpublished) from pecan galls were identified as biovar 1. The correlation between 3-ketolactose production, biovar type, and growth on Schroth et al or New-Kerr selective medium was high. No biovar 2 strains were isolated on Schroth et al medium, and only four biovar 1 strains were isolated on the New-Kerr medium. In this study, fewer strains were isolated on the DIM medium than on the other two media (Table 1).

Virulent strains of both biovars were isolated from the same gall in four of the 18 galls examined. Most pathogenic strains were sensitive in vitro to agrocin 84 (Table 2). Interestingly, eight biovar 1 and four biovar 2 strains were not pathogenic on tomato and *Datura* but were sensitive to agrocin 84. Because agrocin sensitivity is coded by genes on

Table 1. Number of *Agrobacterium* strains isolated from pecan galls on three selective media

3-Ketolactose reaction ^a	Selective medium			Total
	New-Kerr	Schroth et al	DIM	
+	4	24	11	39
-	38	0	4	42

^aThere was a high correlation between the ability of a strain to oxidize lactose to 3-ketolactose and the physiological-biochemical tests that distinguish biovar 1 strains.

Table 2. Number of *Agrobacterium* strains pathogenic and sensitive to agrocin 84 produced by *A. radiobacter* K84^a

Biovar	Pathogens		Nonpathogens	
	Agrocin-sensitive	Agrocin-resistant	Agrocin-sensitive ^b	Agrocin-resistant
1	15	1	8	18
2	2	5	4	32

^aThese data include the four strains isolated earlier by N. W. Schaad.

^bOf 29 agrocin sensitive strains, 12 were not pathogenic.

Table 3. Biological control of representative *Agrobacterium tumefaciens* strains on tomato seedlings by *A. radiobacter* K84

K84	No. infected seedlings (pathogenic strains ^a)			
	B6	GA003	K24	GA1010
Absent	10/10	4/10	10/10	9/10
Present	10/10	2/10	0/10	0/10

^aB6 and GA003 are agrocin-resistant; K24 and GA1010 are agrocin-sensitive. Stem wounds were inoculated with 10^8 cfu/ml of K84, then challenged 24 hr later with 10^7 cfu/ml of the pathogen.

the Ti-plasmid (4), the inability of these strains to infect tomato and *Datura* seedlings may be due to their host specificity (2) or loss of oncogenicity genes but not agrocin-sensitivity genes from the Ti-plasmid. Strain GA003 may also be less virulent because it infected fewer tomato seedlings (Table 3). About 73% of the pathogens isolated from galled pecan trees in Georgia were biovar 1, most of which were sensitive to agrocin 84 (Table 2). Thus, pathogenic biovar 1 strains predominated in this study. In contrast, more biovar 2 than biovar 1 *Agrobacterium* strains were sensitive to K84 in Australia (6) and Oregon (10).

Infection of tomato plants by agrocin-sensitive strain GA1010 isolated from pecan gall was successfully prevented in greenhouse tests with K84 (Table 3). Despite the limited success of biological control of crown gall of peaches by K84 in two other states in the South (1), 94% of the pathogenic biovar 1 strains from pecans in Georgia were agrocin-sensitive, whereas only 29% (two of seven pathogenic strains) of the less prevalent biovar 2 were agrocin-sensitive (Table 2). From the results of these greenhouse tomato tests (Table 3) and because biological control of crown gall is highly correlated with agrocin sensitivity of the pathogen (6), it appears that K84 would prevent the majority of pathogenic agrobacteria from infecting pecans in Georgia. In addition, some pathogenic strains resistant to agrocin 84 in vitro have been prevented from infecting certain hosts by K84 in field experiments (6,10,16).

K84 is used as a preventive and not a curative control agent; therefore, latent

infections are not prevented (9). Latent infections may have occurred on the Jefferson County pecans that were treated with K84 but subsequently developed crown gall. This explanation seems plausible because the pathogenic strains isolated from this sample were sensitive to agrocin 84. Alternatively, K84 may not survive well on roots of pecan or the K84 inoculum may have been adversely affected by other unknown factors after treatment of the young trees (11).

The inhibition of *A. tumefaciens* in vitro by other bacterial antagonists isolated from Georgia indicates that these new antagonists may provide an alternative control for some agrocin 84-resistant pathogenic strains. B6 (biovar 1 and agrocin-resistant) and K24 (biovar 2 and agrocin-sensitive) strains were inhibited in vitro by these new rhizosphere antagonists. The putative antagonists were isolated from a potentially suppressive soil in a young peach orchard in Brooks County where no galled trees were detected and included strains of actinomycetes, fluorescent pseudomonads, *Bacillus*, and *Agrobacterium*. From these antagonists, two actinomycete strains produced large zones of inhibition against the two reference strains and no resistant mutants were observed. Because little success has been noted with other antibiotic-producing antagonists (5,7), these new putative antagonists as well as K84 should be tested under Georgia field conditions for control of crown gall on pecan trees.

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