Crown Gall of Peaches from Maryland, South Carolina, and Tennessee and Problems with Biological Control

R. ALCONERO, Research Plant Pathologist, Agricultural Research, SEA, U.S. Department of Agriculture, Northeast Regional Plant Introduction Station, Geneva, NY 14456

ABSTRACT

ALCONERO, R. 1980. Crown gall of peaches from Maryland, South Carolina, and Tennessee and problems with biological control. Plant Disease 64:835-838.

A high proportion of tumor-inducing *Agrobacterium* isolates from South Carolina (36%) and Tennessee (24%) were resistant to the K84 *A. radiobacter* antagonist commonly used in biological control. Pathogenic forms varied in sensitivity to K84. The weakly sensitive cultures needed a 3:1 or 10:1 ratio of K84 to pathogen for adequate control; these represented approximately 10% of the total pathogenic isolates found in the three states. Apparently latent infections by *A. tumefaciens* were found in nursery trees mailed from Maryland and Tennessee.

Additional key words: sensitivity to agrocins

High incidence of crown gall (*Agrobacterium tumefaciens* (Smith & Townsend) Conn) has recently been reported in peach (*Prunus persica* (L.) Batsch) orchards in South Carolina (1). The distribution, biotype, pathogenicity, and susceptibility to biological control of *Agrobacterium* isolates from peach orchards in South Carolina and from nurseries in Tennessee and Maryland are reported here.

MATERIALS AND METHODS

Thirty gall samples were obtained from diseased trees in South Carolina orchards from August to December 1978, from storage sheds in Tennessee nurseries in October 1978, and from galled peach trees in a nursery in Maryland in August 1978. A total of 56 soil samples were taken in October 1978 from three 10-yr-old commercial peach orchards in Edgefield County, South Carolina, and three nurseries in the McMinnville-Smithville area of Tennessee. The orchard samples were taken approximately 8 cm below the soil surface next to tree trunks, within the row and 1 m between trees, and between rows and 3 m from the trees. The orchards were 17 km apart, and the soil samples were taken within a 90 m^2 area in each orchard.

Of the six nursery fields sampled, four were new to peaches and seeded 2 mo previously and two fields had galls on harvested peach trees the previous year. The soil samples were taken 8 cm below the soil surface from six rows at least 15 m apart per field. The samples were assayed within 48 hr for *Agrobacterium* by plating in selective media. Young healthy peach trees were also transplanted into the soil samples to detect galling.

Isolation and identification. Soil samples were diluted in sterile distilled water (1 g in 100 ml). Galls were surface-sterilized for 5 min in 2.5% sodium hypochlorite and rinsed in sterile distilled water; small internal pieces were macerated in 5 ml of sterile distilled water. Each gall and soil sample was plated on three plates each of the *Agrobacterium*-selective media of Schroth et al (11), New and Kerr (8), Patel (9), and Kado and Heskett medium D1 (2). Single colony isolates were selected at random from the plates after 4 or 5 days of incubation at 24 C. The isolates were transferred to 5-ml glass vials with 3 ml of nutrient broth, incubated 3 days at 24 C, and then plated on King's medium B (4). Cultures that produced fluorescein or were otherwise atypical of *Agrobacterium* were discarded.

The 246 isolates judged typical *Agrobacterium* were selected at random from the three-state collection and tested for 3-ketolactose reaction, growth on 2% NaCl, litmus milk, acid from erythritol and melezitose, alkaline from malonate, tartarate and propionate, and maximum growth temperature as described by Kerr and Panagopoulos (3).

Sensitivity to agrocins. Two methods were used to test the sensitivity of pathogenic *Agrobacterium* isolates to isolate K84 and to 173 other nonpathogenic *Agrobacterium* isolates. The Stonier method used by Moore (6) was compared with a technique that consisted of streaking the antagonist across the center of a yeast mannitol agar (YMA) plate and, after 4-day incubation at 24 C, streaking the test isolates at right angles to it. The test isolates were applied as 48-hr yeast mannitol broth cultures with 3×10^7 colony forming units (cfu) per milliliter and, with a loop, streaking one on either side of the antagonist. Usually five test cultures were evaluated per plate. Reactions were read after 24–48 hr incubation at 24 C. This method is referred to as the streak method to differentiate it from that of Stonier.

Pathogenicity tests. Stems of 5-wk-old Homestead (*Lycopersicon esculentum* Mill.) plants were inoculated with all *Agrobacterium* isolates. A 48-hr-old YMA culture was spread with a loop on the stem surface and introduced into the stem by wounding it lightly with a sterile needle. Three tomato plants were inoculated per culture, kept for 2 wk in a greenhouse at 24–28 C, and then scored for tumor development. The isolates were also tested for their ability to induce tumors on the cut surface of carrot (*Daucus carota* L.) slices by placing a sterile distilled water suspension with 3 $\times 10^7$ cfu/ml of a 48-hr YMA culture on each of three slices. The inoculated slices were incubated in sterile plates with moist filter paper for 2 wk at 24 C and then read for tumor development.

Tests for biological control. *Tomato tests.* All pathogenic isolates were tested for their susceptibility to control by *A. radiobacter* K84 in the greenhouse. The isolates were mixed in sterile distilled water in 1:1 and, in some cases, 3:1, 10:1, and 1:10 proportions of K84 to pathogen. Cultures were grown at 24 C for 48 hr, suspended in sterile distilled water to produce the turbidity that corresponded to the desired cfu/ml, and then mixed to a final total concentration of 4×10^8 cfu/ml. The mixture was injected into the tomato stem by using a 5-ml syringe. Tumor production or control was rated 2 wk after inoculation.

Peach tests. Sixteen soil samples (eight

Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

This work was performed at the Peach Disease Research Unit, USDA, AR, Clemson University, Clemson, SC.

from crown area of trees and eight from between rows) from two of the three South Carolina orchards were sifted to eliminate large rocks and plant debris and placed in 15-cm-diameter plastic pots. The roots of healthy 3-mo-old Lovell peach seedlings grown in individual pots and containing sterilized peat-vermiculite were washed in tap water and wounded by severe cuts and punctures to the root and crown area.

Half of the plants (346) had their root systems and crown immersed for 10 min in 4 L of distilled water with Norbac 84-C (Nortell Laboratories, Inc., Corvallis, OR), a commercial preparation of K84, and then all 692 plants were planted in the soils at three plants per 15-cm-diameter pot. Isolate 37, a peach gall isolate from Maryland, moderately virulent and weakly sensitive to K84, was applied at the base of half the seedlings just after planting (5 ml per plant, 3×10^7 cfu/ml). Treatments consisted of equal numbers of wounded plants (173 plants) treated with Norbac 84-C, with Norbac 84-C and isolate 37, with isolate 37 only, and with no inoculum added. The plants were placed in a random design in a lathhouse and kept for 3 mo at 22–28 C until scored for gall production.

A second test with peaches was made with sterilized soil mixture (sand, soil, peat in equal portions). Lovell peach seedlings were treated as before, planted individually in 15-cm-diameter pots, and the inoculum was applied at the base of the seedling just after planting. The treatments consisted of eight replicates of the K84 isolate alone, of K84 with each test pathogen in a 1:1 and 3:1 ratio, of the test pathogen alone, and of seedlings with no inoculum added. The inoculum suspensions were prepared as described and applied as a 5-ml suspension per plant with a total concentration of 4×10^8 cfu/ml. The plants were placed in a lathhouse as in the first peach trial.

Soils from nurseries and orchards as inoculum. Healthy peach seedlings were planted in soils from the South Carolina orchards and Tennessee nurseries to determine whether the inoculum was soilborne. Six-month-old Lovell peach seedlings individually grown in sterilized 1:3 soil and peat were taken from their pots, the roots washed and wounded, and planted individually in 15-cm plastic pots containing the soil samples. There were 15 pots per soil sample placed in a random design in the greenhouse and the plants were rated for gall development after 3 mo.

Determination of latent infection in nursery trees. Twenty-five 1-yr-old Blake peach trees grafted onto Lovell rootstocks were purchased from each of two nurseries in Tennessee and from a nursery in Maryland. Soon after arrival they were inspected, found to have no galls, and planted individually in 15-cm-diameter plastic pots with 1:1 sterilized soil-sand mixture. The 75 plants were placed approximately 1 m apart in a random design in a lathhouse for 3 mo and then rated for gall development. Special care was taken in the handling of these young trees to avoid wounding or contamination with *Agrobacterium* when they were received and during planting and maintenance in the lathhouse.

RESULTS

Isolation, identification, and distribution of *Agrobacterium* isolates. Of 1,714 bacterial cultures randomly chosen from the four selective media, 659 (38%) were characterized as *Agrobacterium*. Most of the *Agrobacterium* isolates were obtained using the New-Kerr medium (414, 63%) and Schroth et al medium (224, 34%). Biotype determinations of 246 isolates (37%) selected at random from the three-state collection agreed well with the expected biotype responses reported by Kerr and Panagopoulos (3), except that the biotypes could not be differentiated by their growth temperature maxima. All isolates grew at temperatures above 29 C, and some of both biotypes grew at 41 C. The growth responses of the isolates were similar whether they were incubated in a waterbath or in a constant temperature incubator as yeast mannitol broth cultures or as YMA slant cultures.

The proportion of pathogenic to nonpathogenic isolates and the proportion of biotype 1 to biotype 2 isolates varied considerably in the soil and gall samples from the three states (Table 1). In Tennessee, the galls from young trees in the storage sheds had four times more pathogens than nonpathogens, and biotype 2 was twice as numerous as biotype 1. Only 8% of the soil isolates from the Tennessee nurseries were pathogenic, and 48% of the total were biotype 2. Galls from mature trees in South Carolina orchards contained 39% pathogenic isolates, but biotype 2 was found six times more often. Both biotypes were found together in 7% of these galls. The South Carolina orchard soil samples taken away from the trees had only 2% pathogenic isolates, and 7% of the total were biotype 2. The proportion of pathogenic forms increased to 26% in soil samples taken next to orchard tree trunks,

Table 1. Distribution of *Agrobacterium* isolates from Maryland (MD), South Carolina (SC), and Tennessee (TN)

Sample location	Source of *Agrobacterium*		
	Galls	Soil away from tree	Soil near tree
SC (230, 35%)[a]			
Pathogens			
Biotype 1	0	0	2
Biotype 2	26	1	17
Nonpathogens			
Biotype 1	13	42	25
Biotype 2	53	2	49
MD (24, 4%)[a]			
Pathogens			
Biotype 1	0	...	...
Biotype 2	2	...	...
Nonpathogens			
Biotype 1	9	...	...
Biotype 2	13	...	...
TN (400, 61%)[a]			
Pathogen			
Biotype 1	37	2	...
Biotype 2	123	12	...
Nonpathogens			
Biotype 1	25	133	...
Biotype 2	15	53	...

[a] Percentage of all 654 *Agrobacterium* isolated.

Table 2. Sensitivity to K84 by Stonier and streak methods of 308 selected *Agrobacterium* isolates from Maryland (MD), South Carolina (SC), and Tennessee (TN)

Sample location	Sensitivity to K84		
	Resistant	Susceptible	Weakly susceptible
SC (102, 33%)			
Pathogenic			
Biotype 1	2	0	0
Biotype 2	19	33	3
Nonpathogenic			
Biotype 1	29	0	0
Biotype 2	14	2	0
MD (10, 3%)			
Pathogenic			
Biotype 1	0	0	0
Biotype 2	0	0	2
Nonpathogenic			
Biotype 1	1	2	0
Biotype 2	1	4	0
TN (196, 64%)			
Pathogenic			
Biotype 1	1	32	6
Biotype 2	41	74	20
Nonpathogenic			
Biotype 1	13	0	0
Biotype 2	9	0	0

and there were twice as many biotype 2 as biotype 1. Galls from young Maryland trees had a low count of pathogenic forms (12%), and half of the isolates were biotype 2. Carrot disks and tomato stems responded similarly to *Agrobacterium* cultures in pathogenicity tests.

Sensitivity to K84. Table 2 lists the response to K84 of 306 randomly selected *Agrobacterium* isolates tested by the Stonier and streak methods. The isolates represented 44, 35, and 49% of the *Agrobacterium* isolates obtained from South Carolina, Tennessee, and Maryland, respectively. Tennessee is represented by 196 isolates (64%), Maryland by 10 (3%), and South Carolina by 100 (33%). The proportion of pathogenic isolates resistant to K84 in South Carolina was 36%, represented mostly by biotype 2 isolates. Of the pathogenic isolates from Tennessee, 24% were resistant, and they were almost all biotype 2 isolates. Maryland was represented by only two pathogenic isolates, and these were only weakly inhibited by K84 in the plate tests. This type of weak response was found in 31 pathogenic isolates (10%) and mostly in biotype 2. A few nonpathogens were also sensitive to K84 when tested by the Stonier and streak methods. The responses observed in the two plate methods to detect inhibition by K84 were comparable.

Isolates that were sensitive to K84 varied in their sensitivity; this was indicated in Stonier plates by differences in diameter of the inhibition zone (0.2–2.5 cm) and also by a distinct or an indefinite margin of inhibition. In the streak plates the differences in sensitivity were indicated by differences in distance of inhibition of the test cultures from the K84 streak. Figure 1 shows some of these reactions.

The relatively large proportion (27%) of pathogenic isolates that were resistant to K84 prompted the study of 173 nonpathogenic isolates chosen at random from the collection for their possible antagonistic properties. These represented 40% of the nonpathogenic isolates in the collection; 81 were biotype 1, and 92 were biotype 2. They were tested against 21 isolates of both biotypes that were resistant to K84. In the Stonier and streak plate tests, none of the 173 isolates were antagonistic.

Biological control. Table 3 lists the response of peach and tomato plants to inoculation with 10 representative pathogenic isolates applied alone and mixed in various proportions with K84; it also lists the response of the isolates to K84 in the Stonier tests.

In general, the inhibition of the pathogens by K84 in the plate tests was directly related to the responses of the inoculated plants. When there was no inhibition on the plates, there was good development of galls in both peaches and tomatoes, whatever the proportion of antagonist to test culture. When inhibition zones in the Stonier test were 1.5–1.8 cm, there was good control of galling in tomato and some control in peaches. Isolates that had inhibition zones of 0.5–1.4 cm in Stonier plates were weakly controlled at 1:1 and 3:1 (antagonist/pathogen) ratios in peaches and tomatoes. A 10:1 ratio was needed to control these isolates in tomatoes. These isolates gave weak responses in vitro (Table 2). The size and number of galls usually decreased as the ratio of K84 to pathogen increased when these weakly sensitive isolates were tested.

When peach seedlings were planted in samples of orchard soils, none of the seedlings were galled when grown in the soils sampled from between rows of trees, but galling did occur in four samples (50%) taken next to tree trunks. The galling in these four samples varied from 25 to 75%. Applied just before planting, Norbac 84-C completely controlled galling of wounded but healthy peach seedlings in these soils. There was no gall control by Norbac 84-C in any of the soil samples when isolate 37 from Maryland (weakly sensitive to K84) was added at 3×10^7 cfu/ml at the base of each tree immediately after planting. No galls were produced in young seedlings planted in nursery soil samples.

Galls in peach trees from nurseries. The 25 peach trees obtained by mail from each

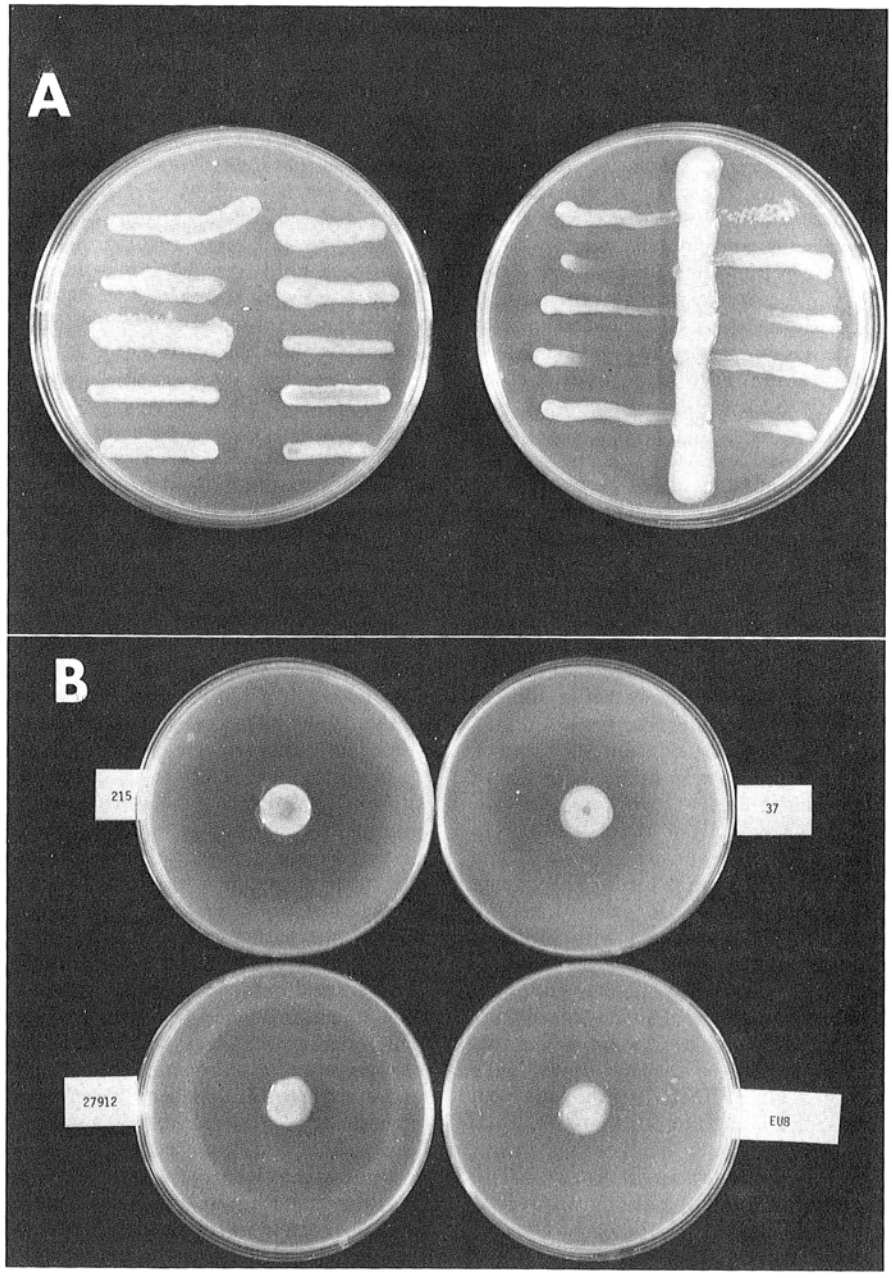

Fig. 1. Sensitivity of *Agrobacterium* isolates to K84. **(A)** Streak method. Left, control plate; right, response of isolates to the K84 streak in the center of the plate. Isolate 37 is the middle isolate on the right side of the plate; the two isolates beside it are resistant to K84. **(B)** Stonier method. Isolate 215 is a sensitive nonpathogenic isolate very susceptible to K84. Isolate ATCC 27912 is pathogenic and very susceptible. Isolate 37 is the least susceptible, and isolate EU8 is resistant to K84.

Table 3. Gall development in tomato and peach plants inoculated with K84 and 10 representative *Agrobacterium tumefaciens* isolates that differed in sensitivity to K84 in Stonier plate tests

Isolate[a]	Tomato[b]					Peach[c]			Stonier inhibition[c]
	Alone	1:10[d]	1:1	3:1	10:1	Alone	1:1	3:1	diam (cm)
EU8	+[f]	+	+	+	+	+	+	+	0
724	+	+	+	+	+	+	+	+	0
T94	+	+	+	+	+	+	+	+	0
T259	+	+	w	w	vw	+	+	w	0.5
37	+	+	+	w	−	+	w	w	1.0
T204	+	+	w	w	−	+	w	w	1.4
585	+	+	−	−	−	+	−	−	1.5
T75	+	+	−	−	−	+	w	−	1.7
T213	+	w	−	−	−	+	w	−	1.8
T364	+	+	−	−	−	+	w	−	1.8

[a] Isolate EU8 was obtained from L. W. Moore; the rest were isolated during these studies.
[b] Results represent one of four tests made and illustrate a common reaction.
[c] Results are from one trial, and the inhibition zone diameters are averages of six trials.
[d] Proportions in inoculum of K84 to pathogen. The mixed inoculum contained 4×10^8 cfu/ml as a distilled water suspension.
[e] Pathogen and K84 streaked as 3×10^7 cfu / ml yeast mannitol broth suspensions.
[f] Response to inoculation: + = good gall development; w = weak gall development, galls smaller; vw = very weak, few and small galls produced; − = no galls.

nursery in Tennessee and Maryland were rated 3 mo after planting. The crown area of 30% of the Maryland trees had galls, 6% from one Tennessee nursery had crown galls, and no galls were found in trees from the other Tennessee nursery. None of the trees had galls when received, suggesting either latent infections or resident *A. tumefaciens* that infected wounds inadvertently made before planting in the sterile potting mix.

DISCUSSION

The selective media of New and Kerr (8) and Schroth et al (11) permitted a better isolation of *Agrobacterium* than did those of Patel (9) and Kado and Heskett (2) even if other microorganisms were not totally excluded. It is apparent that fresh galls from young trees obtained in storage sheds of Tennessee nurseries were better sources of pathogenic isolates than those found in galled seedlings mailed from Maryland or from mature trees in orchards of South Carolina. In previous work I found that peach galls mailed from Tennessee yielded few *Agrobacterium* isolates. The differences in proportion of pathogenic forms and biotypes in galls of mature orchard trees compared with those of young nursery trees suggest that the *Agrobacterium* populations in galls may change considerably. More work is needed to determine if these differences related mainly to the age of the galls sampled. I found that 7% of the galls from orchards contained both biotypes. Such associations could lead to significant genetic interchange among the *Agrobacterium* populations and affect biological control (7).

Soil samples from the nurseries and from the orchards away from the trees yielded few pathogenic forms, and young healthy peach seedlings were not galled when planted in these soils. Galling occurred in soil samples taken next to mature orchard trees where pathogenic populations were higher, and Norbac 84-C completely inhibited gall formation in these soils. The application of isolate 37 at 3×10^7 cfu at the base of the young seedlings after planting in all orchard soils caused galling, even in young healthy seedlings treated with Norbac 84-C. The relatively high concentration of even a moderately virulent isolate such as 37 that is also weakly sensitive to K84 apparently made the commercial preparation ineffective for gall control.

Biological control of crown gall of peaches has had limited success in recent field trials in Pennsylvania and South Carolina (5,10). The reasons for this are not yet clear, but my results suggest that three factors may be relevant in attempts to biologically control crown gall in the region. I found a relatively high proportion (up to 36% in South Carolina) of pathogenic isolates resistant to K84, the most common antagonist used in commercial preparations. In addition, 10% of the pathogenic forms that were sensitive to K84 were only weakly sensitive and needed a 3:1 or 10:1 ratio of K84 to pathogen for adequate control. This weaker sensitivity may partially explain some results from Pennsylvania and elsewhere (7,10) where proportions greater than 1:1 were needed to control crown gall. To my knowledge, differences in sensitivity to K84 and correlation with degrees of success in biological control have not been reported previously. It may be significant that none of the 173 nonpathogenic *Agrobacterium* isolates tested were antagonistic to 21 pathogenic isolates of both biotypes. The 173 isolates represented 40% of all nonpathogenic isolates in the collection and had been taken at random for testing. Antagonism on the root and crown areas under field conditions probably differs considerably from that reported here, however.

The apparent complexity of microbial relations in the biological control of crown gall makes its widely successful application surprising (7). Further improvements in in vitro tests for sensitivity to antagonists may help to better predict the outcome of tests in vivo. In the Stonier and streak plate methods, for example, the assays may not only be for reactions to agrocin 84 but also to other antibiotic factors.

Healthy nursery stock may avoid some galls in peaches in orchards of the region. It remains to be determined whether the resident pathogenic populations of *Agrobacterium* in orchard soils of South Carolina and other states in the southeast can be biologically controlled under field conditions.

ACKNOWLEDGMENTS

I am grateful to G. Freeman and E. Moore for technical assistance.

LITERATURE CITED

1. ALCONERO, R., R. CAMPBELL, J. BRITTAIN, and B. BROWN. 1978. Factors contributing to early decline of peaches in South Carolina. Plant Dis. Rep. 62:914-918.
2. KADO, C. I., and M. G. HESKETT. 1970. Selective media for isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas,* and *Xanthomonas.* Phytopathology 60:969-976.
3. KERR, A., and C. G. PANAGOPOULOS. 1977. Biotypes of *Agrobacterium radiobacter* var. *tumefaciens* and their biological control. Phytopathol. Z. 90:172-179.
4. KING, E. O., M. K. WARD, and D. E. RANEY. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
5. MILLER, R. W., J. A. BRITTAIN, and T. WATSON. 1979. Biological control of crown gall of peach. Fruit South 3:30-32.
6. MOORE, L. W. 1977. Prevention of crown gall on *Prunus* roots by bacterial antagonists. Phytopathology 67:139-144.
7. MOORE, L. W., and G. WARREN. 1979. *Agrobacterium radiobacter* strain 84 and biological control of crown gall. Annu. Rev. Phytopathol. 17:163-179.
8. NEW, P. B., and A. KERR. 1971. A selective medium for *Agrobacterium radiobacter* biotype 2. J. Appl. Bacteriol. 34:233-236.
9. PATEL, M. K. 1926. An improved method of isolating *Pseudomonas tumefaciens* Sm. and Town. Phytopathology 16:577.
10. SCALZA, J., K. D. HICKEY, F. L. LUKEZIC, and R. F. STOUFFER. 1979. Effect of *Agrobacterium radiobacter* var. *radiobacter* strain K84 on gall development by four strains of *A. radiobacter* var. *tumefaciens* in fumigated and non-fumigated soils. (Abstr.) Phytopathology 69:537.
11. SCHROTH, M. N., J. P. THOMPSON, and D. C. HILDEBRAND. 1965. Isolation of *Agrobacterium tumefaciens-A. radiobacter* group from soil. Phytopathology 55:645-647.