Biological Control of Crown Gall in South Africa by *Agrobacterium radiobacter* Strain K84

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ABSTRACT

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In a study on the ability of *Agrobacterium radiobacter* strain K84 to control 82 pathogenic strains of *Agrobacterium*, 36% of 69 South African strains were highly sensitive and 15%, moderately sensitive to agrocin 84 in vitro. Highly sensitive and certain insensitive strains were effectively controlled in vivo after introduction of suspensions at different pathogen:strain K84 ratios into stems of tomato seedlings. Moderately sensitive strains were not effectively controlled in vivo. Studies on tomato seedlings and roots of five other host plant species confirmed that other mechanisms such as physical blockage of infection sites are also involved in biological control.

Additional key words: *Agrobacterium tumefaciens*

Commercial growers of stone fruits and roses in Australia achieve almost complete control of crown gall caused by *Agrobacterium tumefaciens* by dipping propagation material into a suspension of *A. radiobacter* strain K84 before planting (11). Successful biological control with strain K84 has also been reported in several other countries (1–4,6,8,9,14,16–18,20,22,24) including South Africa (15). K84, however, usually fails to control tumor formation on susceptible hosts inoculated with phytopathogenic strains of *Agrobacterium* insensitive to agrocin 84 in vitro (1,12,13,17,19). Furthermore, the discovery in South Africa of three biotypes of *Agrobacterium*, each consisting of different phenotypic groups (7), has emphasized the local need for reevaluating K84 as a biological control agent. This paper reports the efficacy of strain K84 in controlling 69 South African and 13 reference strains of *Agrobacterium*.

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MATERIALS AND METHODS

Bacteria. The phytopathogenic strains of *Agrobacterium* used are listed in a previous paper (7). *A. radiobacter* strain K84 was supplied by A. Kerr, Waite Agricultural Research Institute, University of Adelaide, South Australia.

All inocula were prepared from 48-hr-old cultures on Difco nutrient agar (NA) plates incubated at 28 C. Cultures were diluted in sterile distilled water to absorbances corresponding with suspensions containing the required number of colony-forming units (cfu) per milliliter.

Sensitivity of *Agrobacterium* strains to agrocin 84. The entire surface of individual agar plates of Stonier's (23) medium supplemented with 200 μg of biotin per liter was covered with a disk of sterile cellophane (No. 325, Pretoria Paper Products, Pretoria, South Africa). A suspension of K84 containing about 8×10^8 cfu/ml was spread onto the cellophane. After incubation at 28 C for 72 hr, 35 such disks were transferred to 50 ml of sterile distilled water and kept for 3 days at room temperature. The cells were centrifuged (3,000 *g* for 10 min) and the supernatant, containing agrocin 84, was filter-sterilized through a Millipore membrane filter (pore diameter 0.2 μm) and stored at −10 C.

Single 8-mm-diameter wells were punched in the centers of plates containing Stonier's (23) agar medium, sealed with single drops of the melted medium, and filled with the filtrate

containing the agrocin 84. The plates were incubated at 28 C for 3 hr, then overlaid with phosphate buffer agar (23) seeded with the indicator strain. The double-layer agar plates were incubated at 28 C for 48 hr before being examined for inhibition zones. Each strain was tested twice.

Biological control of *Agrobacterium* strains on tomato seedlings. The phytopathogenic strains of *Agrobacterium* were tested on tomato seedlings (*Lycopersicon esculentum* Mill. cv. Red Khaki). Seed germinated in steam-sterilized sand in a glasshouse at 27 ± 3 C. Six 1-wk-old seedlings were transplanted to each of 15-cm-diameter pots containing a steam-sterilized mixture of peat, loam, and sand (1:1:1). Seedlings received a weekly application of balanced nutrient solution (10).

Two-week-old seedlings were inoculated with different ratios of pathogen:K84. The inoculum of each pathogenic strain was adjusted to about 4×10^8 cfu/ml. A series of suspensions of strain K84 was prepared containing about 4×10^7, 1.3×10^8, 4×10^8, 1.3×10^9, and 4×10^9 cfu/ml. One-milliliter volumes of the suspension of the pathogen were mixed with 1 ml of each of the suspensions of K84 to give pathogen:K84 ratios of 10:1, 3:1, 1:1, 1:3, and 1:10. A suspension of each pathogen mixed with an equal volume of sterile distilled water, as well as sterile distilled water alone, were included as separate controls.

Three seedlings were inoculated per bacterial suspension. All tests were done in triplicate. One drop of suspension was deposited on a tomato seedling stem about 3 cm above soil level and another, between the first and second internodes (i.e., six inoculation points per suspension). Bacteria were introduced into the plants by puncturing the stems through the drops with a sterile needle. The percentage of inoculation points that had tumors after 6 wk was determined for each suspension and the average value of the three replicates calculated.

Biological control of agrocin 84-insensitive *Agrobacterium* strains on other host plant species. Six agrocin 84-insensitive pathogenic test strains of *Agrobacterium* (K28SA, NCPPB 925, Z6SA, 69SA, 2221SA, and Z36SA) and K84 were cultured separately on NA at 28 C for 72 hr. Suspensions of each pathogenic strain in ratios of 1:0, 1:1, and 1:5 of pathogen:K84 were prepared and inoculated onto the same host species from which the strain had originally been isolated: K28SA onto *Chrysanthemum* sp., NCPPB 925 onto *Dahlia* sp., Z6SA onto mazzard cherry (*Prunus avium* L.), 69SA onto Marianna plum (*P. salicina* L.), and 2221SA and Z36SA onto grapevine (*Vitis vinifera* L.). Fifty 2-wk-old seedlings of the ornamental plants and twenty 1-yr-old rooted cuttings of the other hosts were used per treatment. Before inoculation, each plant was root-pruned and wounded further by cutting a longitudinal slit (1–3 cm) along the crown with a sterile knife. Plants were dipped into the appropriate suspensions for 5–10 min and planted immediately in steam-sterilized soil in nursery bags. Plants inoculated with K84 only and wounded, uninoculated plants were included as controls. The soil mixtures and nutrient applications were as described previously. The plants were placed in a lathhouse and rated for the presence of tumors after 3 mo (ornamentals) or 7 mo (other hosts).

RESULTS

Agrocin 84 sensitivity. Forty-one of all strains tested were sensitive to agrocin 84 (Fig. 1). Thirty-one of these were highly sensitive and 10, moderately sensitive. Thirty-six percent of the South African

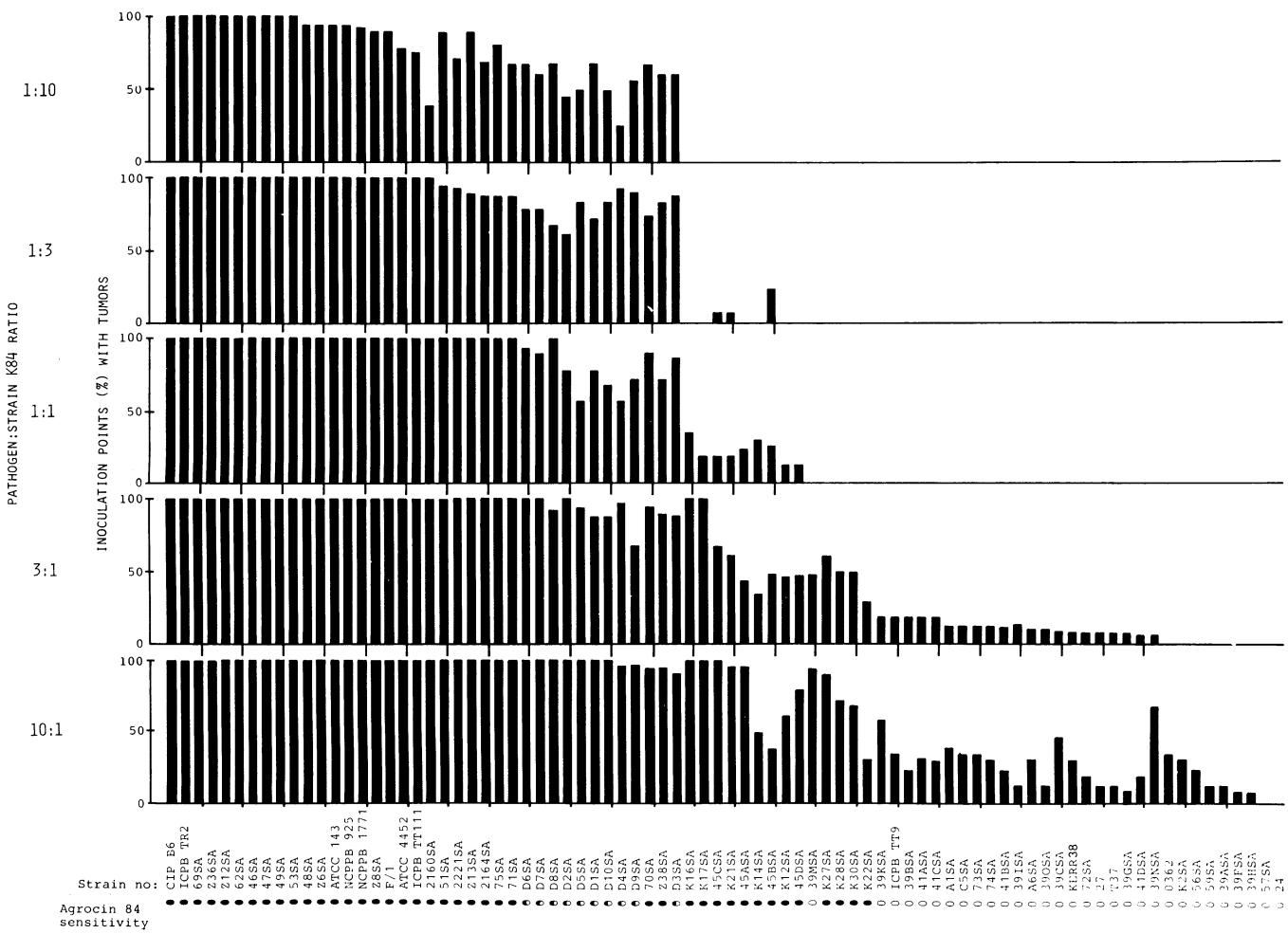

Fig. 1. Biological control of 82 strains of *Agrobacterium* by *A. radiobacter* strain K84 on tomato seedlings inoculated with suspensions containing different pathogen:K84 ratios. Sensitivity of test strains to agrocin 84 in vitro: ● = insensitive, ◐ = moderately sensitive (diameter of inhibition zones 1.5–2 cm), and ○ = highly sensitive (zones 3.5–4.6 cm).

Table 1. Biological control of six agrocin 84-insensitive strains of *Agrobacterium* by *A. radiobacter* strain K84 on roots of five host plant species

Inoculation treatment					
Pathogen strain	Pathogen:K84 ratio	Host plant	No. of plants examined	Plants with tumors (%)	χ^2 (2 df)[a]
K28SA	1:0	*Chrysanthemum* sp.	32	53.1	
	1:1		37	24.3	
	1:5		32	25.0	7.95*
NCPPB 925	1:0	*Dahlia* sp.	37	94.6	
	1:1		46	87.0	
	1:5		43	65.0	12.90**
Z6SA	1:0	*Prunus avium*	17	100.0	
	1:1		19	100.0	
	1:5		20	50.0	21.91**
69SA	1:0	*P. salicina*	17	29.4	
	1:1		20	0.0	
	1:5		20	0.0	12.90**
2221SA	1:0	*Vitis vinifera*	17	82.3	
	1:1		17	70.6	
	1:5		20	60.0	2.20 ns
Z36SA	1:0	*V. vinifera*	16	56.3	
	1:1		20	60.0	
	1:5		18	38.9	1.89 ns

[a] Chi-square test for differences between binomial proportions (21); * = significant at $P = 0.05$, ** = significant at $P = 0.01$, and ns = not significant.

strains were highly sensitive and 15%, moderately sensitive.

Biological control on tomato seedlings. Tumor development after inoculation with suspensions containing different ratios of pathogen:K84 is recorded in Figure 1. At a ratio of 1:1, 47 strains caused tumors. Of these, 27 caused tumors at each inoculation point (100%), whereas lower values (11–94%) were recorded for the other 20 strains. Strains highly sensitive to agrocin 84 in vitro did not cause tumors at a ratio of 1:1. Inoculation points with tumors recorded for the 10 moderately sensitive strains ranged between 56 and 95%. Four strains insensitive to agrocin 84 (K27SA–K22SA) failed to cause tumors at this ratio.

At a 1:10 ratio, 31 strains insensitive to agrocin 84 (K16SA–45DSA and K27SA–K22SA) induced no tumors, or the percentages of tumors were reduced to 37–94% (48SA–71SA, 70SA, and Z38SA).

Twenty-nine strains highly sensitive to agrocin 84 (39MSA and 39KSA–39HSA) were not completely inhibited at a ratio of 10:1. Two other strains (57SA and 24), however, caused no tumors at this ratio.

Fifty-five percent of the South African strains failed to cause tumors when mixed with K84 (1:10 ratio). Thirty-four percent of these were agrocin 84-insensitive.

Biological control on other host plant species. The percentages of plants with tumors recorded for the pathogenic strains alone ranged between 100% for strain Z6SA on *P. avium*, and 29.4% for strain 69SA on *P. salicina* (Table 1). Lower percentages of affected plants were recorded for strains K28SA, NCPPB 925, and 2221SA applied at a pathogen:K84 ratio of 1:1. Strain 69SA caused no tumors at ratios of 1:1 or 1:5. At a 1:5 ratio, lower percentages of affected plants were recorded for strains NCPPB 925, Z6SA, 2221SA, and Z36SA compared with the 1:1 ratio. No tumors developed on control plants.

DISCUSSION

The feasibility of biological control of crown gall by *A. radiobacter* K84 in South Africa was investigated by comparing the antagonistic activities of K84 against 69 South African and 13 reference strains of *Agrobacterium*.

Only 51% of the South African strains were sensitive to agrocin 84 in vitro. Highly sensitive strains were effectively controlled in vivo if suspensions containing pathogen:K84 ratios of 1:1, 1:3, or 1:10 were introduced into stems of tomato seedlings. Some highly sensitive strains were also effectively controlled if ratios of 3:1 or 10:1 were applied; however, strains moderately sensitive to agrocin 84 were not effectively controlled at a ratio of 1:10. This is in contrast to results of others (1,12).

Our findings confirmed reports (16–18,20) that some strains insensitive to agrocin 84 can be controlled on plants by K84. Thirty-nine percent of the South African agrocin 84-insensitive strains were effectively controlled by K84 on tomato seedlings (1:10 ratio, Fig. 1). Furthermore, K84 significantly reduced the percentages of plants with tumors caused by agrocin 84-insensitive strains on roots and crowns of different host plant species (Table 1).

Our data agree with those of Cooksey and Moore (5), suggesting that besides inhibition by agrocin 84, mechanisms such as physical blockage of infection sites are also involved in biological control. Apparently, the host plant affects the mechanism of control. For example, agrocin 84-insensitive strain K28SA was completely suppressed by K84 on tomato (Fig. 1) but not on *Chrysanthemum* plants (Table 1). Furthermore, Moore (18) found that strain K84 effectively controlled a mixture of four agrocin 84-insensitive strains of *A. tumefaciens* on pear but not on apple seedlings.

Studies on tomato seedling stems and roots of other host plants showed that crown gall can be controlled fairly effectively by K84 in South Africa. Since agrocin 84-insensitive strains of *Agrobacterium* were not completely suppressed by K84 in all instances, this study emphasizes the need to search for additional antagonists effective against all or most crown gall agrobacteria.

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