APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1989, p. 741–746
0099-2240/89/030741-06$02.00/0

Vol. 55, No. 3

Evidence of Biological Control of *Agrobacterium tumefaciens* Strains Sensitive and Resistant to Agrocin 84 by Different *Agrobacterium radiobacter* Strains on Stone Fruit Trees

MARÍA M. LÓPEZ,* MARÍA TERESA GORRIS, CARMINA I. SALCEDO, ANA M. MONTOJO, AND MARCELA MIRÓ

Instituto Valenciano de Investigaciones Agrarias, I.V.I.A. Apartado Oficial, 46113, Moncada, Valencia, Spain

Received 15 August 1988/Accepted 27 December 1988

The effectiveness of *Agrobacterium radiobacter* K84, 0341, and a K84 non-agrocin-producing mutant (K84 Agr$^-$) in biological control of crown gall on rootstocks of stone fruit trees was determined in three experiments. In experiment 1, K84 and 0341 controlled crown gall on plum plants in soil inoculated with two strains of *Agrobacterium tumefaciens* resistant to agrocin 84. In experiment 2, K84 controlled crown gall on peach plants in soils inoculated with strains of *A. tumefaciens* sensitive or resistant to agrocin 84 or with a mixture of both. However, the effectiveness of K84 was higher against the sensitive strain than against the resistant strain. There was a residual effect of K84 from one year to another in soil inoculated with the sensitive strains. In experiment 3, K84 and K84 Agr$^-$ controlled crown gall on plum and peach plants in soils inoculated with strains of *A. tumefaciens* sensitive or resistant to agrocin 84. The control afforded by K84 was higher than that provided by K84 Agr$^-$ against the sensitive strain but was similar against the resistant strain.

Successful biological control of crown gall has been reported in many countries (8, 23, 24). The method is being applied commercially, using various preparations of strain K84 of *Agrobacterium radiobacter* (23). Seeds, cuttings, or plants are dipped into suspensions of K84 before being planted in soils presumably infested with *Agrobacterium tumefaciens* (11, 12).

According to Kerr and Htay (13), biological control is due to the production of a bacteriocin, agrocin 84, by the controlling organism. In agreement with this, isolates of *A. tumefaciens* sensitive in vitro to agrocin 84 have been controlled by K84 (8, 13, 23), although in some cases a breakdown of the biological control has been observed (23, 27). Other authors, however, also observed a reduction in infection when using K84 against a mixture of both agrocin 84-sensitive and -resistant strains (22, 23, 29) or even against certain resistant strains (2, 5, 35). In other cases, no control was obtained by using K84 against agrocin 84-resistant isolates (1, 23, 27). Cooksey and Moore (3) observed that either K84 or a K84 non-agrocin-producing mutant controlled crown gall on tomato plants inoculated with an agrocin-resistant strain. Their data suggest that, in addition to production of agrocin 84, other mechanisms may be involved in biological control by K84.

In Spain, crown gall is widespread and constitutes a potential threat to deciduous fruit trees and rose nurseries and plantations. A study of the characteristics of 225 Spanish isolates of *A. tumefaciens* has shown that 14.3% of the isolates from biovar 1 and 23.7% from biovar 2 were resistant to agrocin 84 (19). The relative abundance of agrocin 84-resistant strains and the different results obtained by several authors (1, 5, 24, 27, 35) using strains exhibiting these characteristics led us to compare the effectiveness of different *A. radiobacter* strains for biological control of crown gall in soils artificially infested with *A. tumefaciens* strains sensitive or resistant to agrocin 84. Evidence of the influence of other possible mechanisms of control not related to agrocin 84 production is presented here.

MATERIALS AND METHODS

Bacterial strains. (i) ***A. radiobacter.*** In experiment 1, *A. radiobacter* K84 and 0341 were used. Strain K84 was isolated in Australia and produces agrocin 84 (12, 13). Strain 0341 produces agrocin 84 and is an avirulent biovar 1 strain originating from the recombination of a pathogenic strain with K84 (28). It was kindly supplied by C. G. Panagopoulos, Faculty of Agriculture, Athens, Greece.

In experiment 3, a K84 mutant which no longer produces agrocin 84 (K84 Agr$^-$) was used. It was obtained by treating K84 with mitomycin C (3) and was kindly provided by L. W. Moore, Oregon State University, Corvallis, Oreg.

(ii) ***A. tumefaciens.*** In experiment 1, *A. tumefaciens* 1001 from Collection Nationale de Bactéries Phytopathogènes, Angers, France (ex TR 104 Starr) and 2437 NCPPB (ex B6 A. C. Braun) were used. They were isolated from apple and tomato tumors, respectively. Both are biovar 1 and utilize octopine and mannopinic acid, according to the methods of Lippincott et al. (17) and M. T. Gorris, M. M. López, and C. Morente (Abstr. 4th Congreso Nacional de Fitopatología, p. 2, 1985). According to the method of Stonier (34), these strains were resistant to agrocin 84 in vitro and were not bacteriocin producers.

In experiment 2, *A. tumefaciens* 014 from Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia, Spain (IVIA) and 020 IVIA were used to inoculate soil. They were isolated from peach tumors from nurseries in Zaragoza and Valencia, respectively. Both are biovar 2 and utilize nopaline but not octopine or mannopinic acid. Isolate 020 is sensitive to agrocin 84 (S), and isolate 014 is resistant (R).

In experiment 3, *A. tumefaciens* 251-1 IVIA and 251-2 IVIA were used to inoculate soil. Both strains were isolated from a plum tumor and were selected as purified colonies off the same isolation plate. Strain 251-1, biovar 1, is sensitive to agrocin 84 (S), and strain 251-2, biovar 2, is resistant (R). Both utilize nopaline but not octopine or mannopinic acid.

* Corresponding author.

Field trials. (i) Soil inoculation. All experiments were performed in concrete containers (5 by 2 by 0.5 m) that were filled with soil typical of the area (clay loam, calcareous, pH 8.5). Soil in each container was inoculated with the *A. tumefaciens* strains mentioned above by adding 40 liters of water with 1 liter of bacterial suspension (ca. 10^9 CFU/ml). Flood irrigation was done after soil inoculation. The final concentration in soil was about 10^6 CFU/g. *A. tumefaciens* could not be isolated from soil before inoculation.

(ii) Plant treatments. In the three experiments, dormant plants (1 year old) were used. They came from a commercial nursery. They were wounded superficially by insertion of a knife in the crown, and roots were cut at 10 to 20 cm immediately before treatment.

Control plants were dipped in a suspension of 5 kg of peat in 10 liters of water.

Plants treated with *A. radiobacter* K84, 0341, or K84 Agr^- were dipped in a suspension of peat preparation of K84, 0341, or the mutant, prepared by mixing 5 kg of inoculated peat with 10 liters of water by the method of López et al. (20). The final bacterial concentration on the peat inoculum was about 10^9 CFU/g.

Plants treated with Copac were dipped in a suspension of 50 ml of Copac (BASF, Ludwigshafen, Federal Republic of Germany) and 50 ml of Propiofan glue (BASF) in 5 liters of water.

Experimental design. In experiment 1, four containers were used, and 110 myrobolan plum trees (*Prunus cerasifera* Ehrh.) and 110 M-9 apple trees (*Malus pumila* Mill.) were used for each of the following treatments: control, K84, 0341, and Copac. Plants for each treatment were placed in a different container.

Experiment 2 was conducted over 2 consecutive years in six containers. In the first year, three soil infection treatments, one with strain 020 (S), another with strain 014 (R), and the third with a 1:1 mixture of both of them (S + R) were done. For each soil treatment two containers were used; one was planted with 200 control peach seedlings (*Prunus persica* Batsch.), and the other was planted with 200 K84-treated peach seedlings. In the second year, the soils in the containers from the first year were used after removal of the plants. Keeping the soil from the previous year, each container was divided in half; one half was planted with 100 control peach seedlings, and the other was planted with 100 K84-treated peach seedlings. In this study, the effectiveness of K84 remaining in the soil from the treatment of the previous year was determined and compared with that of the new treatment.

In experiment 3, two soil infection treatments were done, one with strain 251-1 (S) and the other with strain 251-2 (R). Three containers were used for each of the two soil treatments. One container was planted with control plants, another was planted with K84-treated plants, and the third was planted with the K84 Agr^--treated plants. In each container, 100 myrobolan plum trees and 100 peach seedlings were planted.

In the three experiments, plants were grown for 10 to 12 months. In spring and summer, 2 kg of ammonium nitrosulfate (26% N) was added to each container and plants were sprayed with 2.4% Fe three times. Flood irrigation was used. In winter, the plants were dug out and scored for galling. The number, position, and weight of tumors per plant and the number of plants with galls were noted for each treatment.

Statistical analysis. Analysis of variance was performed, and data of percentages of infected plants and of plants with one tumor were transformed by arcsin $\sqrt{p/100}$ (p = percentage of infected plants). Data of mean weight were not transformed. The means were compared by using the Duncan multiple range test.

TABLE 1. Effectiveness of crown gall biocontrol on myrobolan plum and M-9 apple rootstocks planted in soil artificially inoculated with *A. tumefaciens*[a]

Host	Treatment[b]	% Plants with galls	Transformed means of data[c]	Mean fresh wt (g) of galls/plant
Plum	Control	39.19	0.64a	7.36b
	K84	2.73	0.09b	0.18c
	0341	1.82	0.06b	0.40c
	Copac	46.08	0.74a	17.81a
	EMS (df)		0.038 (40)	53.07 (40)
Apple	Control	0.00		0.00
	K84	2.75		0.11
	0341	1.82		0.41
	Copac	0.93		0.41

[a] Soil was inoculated with *A. tumefaciens* 2437 and 1001, both resistant to agrocin 84 (ca. 10^6 bacteria per g of soil). In the last two columns, numbers followed by the same letter are not significantly different ($P = 0.05$) according to the multiple range test of Duncan.

[b] Eleven rows with 10 plants each were planted per treatment. Control plants were dipped in a suspension of 5 kg of peat in 10 liters of water. Other plants were dipped in a suspension of 5 kg of peat preparation of *A. radiobacter* K84 or 0341 in 10 liters of water or were dipped in 50 ml of Copac (BASF) and 50 ml of Propiofan D6 (BASF) in 5 liters of water. EMS, Error mean square; df, degrees of freedom.

[c] Means of data transformed by arcsin $\sqrt{p/100}$ (p = percentage of plants with galls).

Bacteriological methods. To detect the appearance of recombinants described in other experiments (27), bacteria from K84- and 0341-treated plants and from untreated controls in experiment 1 and from K84-treated plants in experiment 2 were reisolated by plating tumor tissue macerates from the galls in duplicate plates. Selective medium (25, 30) was used. Twenty galls from each treatment were analyzed. From each isolation plate, up to five isolated typical colonies were randomly selected, purified, and characterized for biochemical and pathogenicity tests. The pathogenic reisolates were also tested for sensitivity to agrocin 84 and for bacteriocin production and were compared with the original strains. Methods for all these tests were as described by Kerr and Panagopoulos (14).

RESULTS

Experiment 1. Results of tests of K84 effectiveness on plum and apple are shown in Table 1. The incidence of trees with galls was higher in plum than in apple. The incidence of disease in apple trees was very low for all treatments, and for this reason the data obtained were not analyzed statistically.

The results obtained for plum trees show the effectiveness of K84 and 0341 against two *A. tumefaciens* strains which are resistant to agrocin 84 and which utilize octopine. No differences in the percentage of diseased plum plants were found with control and Copac treatments; however, there were significant differences between those plants and those treated with K84 or 0341, showing evidence of biocontrol. No significant differences were found between plants treated with K84 and those treated with 0341. The mean weight of tumors per plant was significantly higher for plants treated with Copac or for the controls than for plants treated with K84 or 0341. No differences were observed between the latter two treatments, but the mean weight of tumors on

TABLE 2. Effectiveness of crown gall biocontrol on peach seedlings planted in soil artificially inoculated with *A. tumefaciens*

Inoculum[a]	Treatment[b]	% Plants with galls	Transformed means of data[c]	Mean fresh wt (g) of galls/plant	% Diseased plants with one gall	Transformed means of data[d]
020 (S)	Control	91.92	1.35	30.21	23.03	0.47
	K84	17.39	0.42	0.11	85.84	1.32
014 (R)	Control	94.43	1.42	52.82	20.85	0.42
	K84	46.93	0.75	1.76	52.28	0.81
020 (S) + 014 (R)	Control	93.78	1.37	32.33	28.71	0.53
	K84	41.67	0.70	1.66	67.04	1.04
	EMS (df)		0.027 (54)	137.44 (54)		0.086 (54)

[a] Soil was inoculated with agrocin 84-sensitive *A. tumefaciens* 020 (S), agrocin 84-resistant 014 (R), or the combination of both (1:1) (ca. 10^6 bacteria per g of soil in all cases).

[b] Ten rows with 20 peach seedlings each were planted per treatment. Control seedlings were dipped in a suspension of 5 kg of peat in 10 liters of water. K84-treated seedlings were dipped in a suspension of 5 kg of peat preparation of *A. radiobacter* K84 in 10 liters of water. EMS, Error mean square; df, degrees of freedom.

[c] Means of data transformed by arcsin $\sqrt{p/100}$ (p = percentage of plants with galls).

[d] Means of data transformed by arcsin $\sqrt{p/100}$ (p = percentage of diseased plants with one gall).

plants treated with Copac was significantly higher than that of plants given the other treatments.

The characteristics of the 123 pathogenic *Agrobacterium* isolates from tumors found in control plants and in plants treated with Copac were consistent with characteristics of the inoculated strains. For unknown reasons, no virulent agrobacteria were recovered from plants treated with K84 or 0341.

Experiment 2. (i) First year. Table 2 shows the results obtained in the first-year study. Treatment with strain K84 significantly reduced the percentage of plants with galls and the mean weight of tumors per plant on plants growing in soil inoculated with strain 020 (S) or 014 (R) or both. The reduction of disease obtained in the soil inoculated with the sensitive strain was significantly higher than that obtained in the soil inoculated with the resistant strain. In the plants with galls, the percentage of plants with only one tumor was significantly higher for the K84-treated plants than for the control plants.

The study of 158 isolates of *A. tumefaciens* obtained from tumors of plants treated with K84 showed that all of the isolates obtained from plants growing in soil inoculated with strain 020 (S) were sensitive to agrocin 84. Those from plants growing in soil inoculated with strain 014 (R) were resistant

TABLE 3. Effectiveness of crown gall biocontrol on peach seedlings planted in soil artificially inoculated the year before with *A. tumefaciens*

Previous treatment and inoculum[a]	Second-year treatment[b]	% Plants with galls	Transformed means of data[c]	Mean fresh wt (g) of galls/plant	% Diseased plants with one gall	Transformed means of data[d]
Control						
020 (S)	Control	45.60	0.61	3.73	59.11	0.90
	K84	3.87	0.07	0.05	50.00	0.79
014 (R)	Control	73.36	0.97	19.59	44.63	0.73
	K84	8.14	0.19	0.18	75.00	1.18
020 (S) + 014 (R)	Control	84.91	1.01	19.57	35.72	0.61
	K84	13.25	0.28	0.17	95.80	1.49
K84						
020 (S)	Control	11.45	0.29	0.61	54.12	0.86
	K84	1.82	0.04	0.02	50.00	0.79
014 (R)	Control	75.71	0.98	13.17	43.83	0.72
	K84	14.54	0.28	0.35	95.80	1.49
020 (S) + 014 (R)	Control	67.40	0.90	2.59	60.31	0.90
	K84	18.60	0.29	0.31	70.00	1.12
	EMS (df)		0.053 (120)	8.34 (120)		0.15 (85)

[a] Soil was inoculated the previous year with agrocin 84-sensitive *A. tumefaciens* 020 (S), agrocin 84-resistant 014 (R), or the combination of both (1:1) (ca. 10^6 bacteria per g of soil in all cases). Plants of the previous-year study were treated or untreated with K84 as indicated. They were removed before the new planting.

[b] Ten rows with 11 peach seedlings each were planted per treatment. Control seedlings were dipped in a suspension of 5 kg of peat in 10 liters of water. K84-treated seedlings were dipped in a suspension of 5 kg of peat preparation of *A. radiobacter* K84 in 10 liters of water. EMS, Error mean square; df, degrees of freedom.

[c] Means of data transformed by arcsin $\sqrt{p/100}$ (p = percentage of plants with galls).

[d] Means of data transformed by arcsin $\sqrt{p/100}$ (p = percentage of diseased plants with one gall).

TABLE 4. Effectiveness of crown gall biocontrol on peach and plum plants planted in soil artificially inoculated with *A. tumefaciens*

Host	Inoculum[a]	Treatment[b]	% Plants with galls	Transformed means of data[c]	Mean fresh wt (g) of galls/plant	% Diseased plants with one gall	Transformed means of data[d]
Peach	251-1 (S)	Control	84.42	1.23	17.85	28.50	0.48
		K84	11.30	0.28	0.80	100.00	1.57
		K84 Agr	43.30	0.78	2.54	70.80	1.08
	251-2 (R)	Control	97.63	1.50	53.90	4.60	0.14
		K84	4.12	0.10	0.04	100.00	1.57
		K84 Agr$^-$	17.49	0.31	0.35	69.60	1.10
Plum	251-1 (S)	Control	85.00	1.22	20.08	31.20	0.58
		K84	7.00	0.16	0.55	70.00	1.10
		K84 Agr	79.30	1.18	13.90	48.50	0.77
	251-2 (R)	Control	85.86	1.23	40.18	7.26	0.21
		K84	8.22	0.20	0.75	71.40	1.12
		K84 Agr$^-$	10.11	0.26	0.16	96.40	1.50
		EMS (df)		0.05 (108)	85.28 (108)		0.13 (83)

[a] Soil was inoculated with agrocin 84-sensitive *A. tumefaciens* 251-1 (S) or agrocin 84-resistant 251-2 (R) (ca. 10^6 bacteria per g of soil).
[b] Ten rows with 10 plants each were planted per treatment. Control plants were dipped in a suspension of 5 kg of peat in 10 liters of water. Other plants were dipped in a suspension of 5 kg of peat preparation of *A. radiobacter* K84 in 10 liters of water or in a suspension of 5 kg of peat preparation of a K84 non-agrocin-producing mutant. EMS, Error mean square; df, degrees of freedom.
[c] Means of data transformed by arcsin $\sqrt{p/100}$ (p = percentage of plants with galls).
[d] Means of data transformed by arcsin $\sqrt{p/100}$ (p = percentage of diseased plants with one gall).

to agrocin 84. Of the isolates from plants growing in soil inoculated with the mixture of both strains, 23% were sensitive to agrocin 84 and 77% were resistant. All of the *A. tumefaciens* strains which were reisolated were biovar 2, like the inoculated strains, and did not produce agrocin 84.

(ii) Second year. From the data in Table 3, two types of residual effects can be studied: the effect of *A. tumefaciens* and that of K84. The residual effect of *A. tumefaciens* was observed by comparing the incidence of the disease in the controls for the first year (Table 2) and the second year (three first-year soil inoculation treatments in Table 3). The incidence of plants with galls decreased drastically in the second year in the soil inoculated with strain 020 (S) (91.92 versus 45.6%). The decrease was slight in the other soils.

The results of the second year confirmed the effectiveness of K84 observed in the first year. In the study overall, there was a significant reduction in the percentage of plants with galls and in the weight of tumors per plant in plants treated with K84 compared with the controls.

The aim of this experiment was also to observe the residual effect of K84, i.e., the effectiveness in biocontrol of the K84 population that continued to live in the soil after removal of the plants treated the previous year. In this study overall, no significant differences were observed in either the percentage of plants with galls or the percentage of plants with one tumor between plants from soil in which controls had been grown the previous year and plants from soil in which K84-treated plants had been grown the previous year. However, the weight of tumors per plant was significantly lower on plants from soil in which K84-treated plants had been grown the year before. This suggests that the residual K84 was more effective in reducing tumor size than in reducing the incidence of disease, considering the overall study. On the other hand, considering the residual effect of K84 on the control plants of the second year, there was a significant reduction of the percentage of plants with galls for soils inoculated with 020 (S) and soils in which K84-treated plants had been grown the previous year (11.45 versus 45.6%), whereas no or a lower residual effect was observed in soils inoculated with 014 (R) or with the mixture of both strains.

The study of 57 isolates of *A. tumefaciens* obtained from tumors of K84-treated plants showed that all the isolates had characteristics similar to those of the inoculated strains; i.e., they were sensitive or resistant to agrocin 84 and were biovar 2. All the isolates from plants growing on soil inoculated the previous year with a mixture of 020 (S) and 014 (R) were resistant to agrocin 84, a fact that seems to confirm good survival of 014 (R) in the soil. No isolates producing agrocin 84 were obtained.

Experiment 3. Table 4 shows the effectiveness of K84 in peach and plum plants. K84 and K84 Agr$^-$ were effective against *A. tumefaciens* strains sensitive or resistant to agrocin 84. A significant reduction in the percentage of diseased plants was found in K84-treated plants and in plants treated with K84 Agr$^-$, compared with the control. The percentage of plants with a single tumor was higher in K84- or K84 Agr$^-$-treated plants than in the control. The effectiveness of K84 was significantly higher than that obtained with K84 Agr$^-$. However, K84 and K84 Agr$^-$ reduced significantly the mean weight of tumors per plant, and no differences were observed in plants given these two treatments.

When both hosts grown in the soil inoculated with the 251-1 (S) strain were examined, significant differences were found in the percentage of plants with galls among the control plants and plants treated with K84 or K84 Agr$^-$. K84 was significantly more effective than K84 Agr$^-$ in reducing the incidence of plants with galls and the weight of tumors per plant. However, in the soil inoculated with the 251-2 (R) strain, since sensitivity to agrocin 84 did not play any role, significant reduction of the disease was observed in plants treated with K84 or K84 Agr$^-$, but there was no difference between plants given these two treatments. The incidence of disease was similar in peach and plum plants except for those treated with K84 Agr$^-$; i.e., no difference between controls and between plants treated with K84 was found.

DISCUSSION

The results obtained in experiments 1, 2, and 3 show that the soil inoculation method and the strains used in the assays produced high levels of crown gall infection, except in apple.

In experiment 1, a higher sensitivity to *A. tumefaciens* was observed in myrobolan plum than in apple M-9. Considering that apple M-9 is very sensitive to crown gall in natural conditions (9), it is possible that the strains used had a low affinity for or low virulence with apple. Unsuccessful inoculations of apple have been reported by other authors (10, 15, 18, 36).

Results from these experiments showed the low effectiveness of Copac treatment for crown gall control. This copper compound, however, gave satisfactory control on apple trees (9), although it was used at higher doses than that applied in this assay. Other authors have observed no efficacy of other copper compounds in controlling crown gall on stone fruit trees (22, 32).

No differences were found between strains K84 and 0341. In contrast, Panagopoulos et al. (28) stated that the biological control efficiency of 0341 was clearly higher than that of K84, although entire crown gall prevention was not achieved. In our study, the lack of conjugation ability of 0341 in relation of K84 was not studied.

Experiment 2 showed that one treatment with K84 was not sufficient to achieve good biological control through the following year. The population or infectivity of *A. tumefaciens* seemed to decrease from one year to another when plants were treated with K84, but only when the agrocin 84-sensitive strain was involved. In general, it seems that a high population of K84 is necessary around the roots to achieve efficient control. For this reason, treatments with K84 in the planting row proved less effective than treatment of cuttings immediately before planting (20).

The effectiveness of K84 in controlling crown gall was observed for different hosts growing in soils inoculated with *A. tumefaciens* strains which utilize octopine or nopaline and which are sensitive or resistant to agrocin 84. The strains came from international collections or were isolated in Spain. In all cases, a reduction was observed in the number of plants with tumors and in the weight and number of tumors per plant with both sensitive and resistant strains. It is generally accepted that resistant strains are those not inhibited by K84, according to the classical method of Stonier (34). However, the results of other authors (2, 4, 24, 33, 35) suggest that the classification of *A. tumefaciens* strains as sensitive or resistant to agrocin 84 depends on the technique used and that the strains of *A. tumefaciens* may not be sensitive or resistant in absolute terms. Strains 2437 and 1001 were resistant by the method of Stonier and by the modified test of Dhanvantari (4), whereas 014 (R) and 251-2 (R) were resistant on Stonier medium but showed a small zone of inhibition when tested by the modification of Dhanvantari.

Our use of infested soil simulated more closely the inoculum conditions found in nature for *A. tumefaciens*. Natural soil inoculum represents a sharp contrast to the more drastic method of dipping or spraying seedling roots directly with *A. tumefaciens*. The latter method tends to overwhelm the system because the roots are exposed to large populations of *A. tumefaciens*, increasing the probability of intimate contact with wounded plant cells. In contrast, infested soil provides a lower inoculum pressure, thus allowing more subtle mechanisms of antagonism to operate, which may explain why a positive effect was observed with the K84 Agr^- strain.

Our results showed control of agrocin-resistant *A. tumefaciens* strains and proved that other factors contribute to the efficacy of biological control besides agrocin 84 production and the sensitivity or resistance of *A. tumefaciens* to the bacteriocin. Other authors (7, 31) reported that production of agrocin 84 is still the primary mechanism of biological control by K84.

Biological control obtained on peach and plum trees by using K84 Agr^- might be explained if this strain, as K84, colonizes the roots and likely sites of infection, which would prevent a subsequent attack by *A. tumefaciens*. *A. tumefaciens* will likely succeed best in infecting a wound which is poorly colonized by other microbes. This idea is supported by the observation that a greater incidence of plants with one tumor occurred among those treated with K84 or with K84 Agr^- than among the untreated controls. A blockage or competition for infection sites (16) had been suggested by some authors to explain biocontrol of resistant isolates in some studies (3, 5, 6). *A. radiobacter* has proved a good colonizer of the root system (23, 26), but the direct attachment of K84 to the plant cells has not yet been demonstrated.

Substrate competition between K84 and *A. tumefaciens* in soil might explain the percentages of diseased plants found in plum and peach plants, using the K84 Agr^- strain. The idea is that a certain competition in the soil between *A. tumefaciens* and K84 or K84 Agr^- might take place if they are of the same biovar and use the same substrates. This is true in soil inoculated with 251-2 (R), which is biovar 2, like K84 and K84 Agr^-. Since the initial population of K84 and K84 Agr^- is larger than that of *A. tumefaciens* in the root system, a certain competition between them for the same substrates may affect the proliferation of the virulent strain. Conversely, strain 251-1 (S) is biovar 1, and hence there might be less competition from the protecting strains. In this case, the *A. tumefaciens* population will increase and will not be properly controlled by the K84 Agr^-. The competition would not be so clearly observed when K84 is acting against sensitive strains, since the more rapid effect of agrocin 84 could mask such competition.

A serious problem affecting the success of biological control is the presence of pathogenic recombinants observed in other experiments (27), but they have not been detected in the plants treated with K84 in our studies. The likely nonpathogenic recombinants were not studied because they did not directly affect the efficiency of biocontrol.

The results obtained with strain K84 in stone fruit trees and in other species (19–21) suggest the convenience of using K84 until new strains become available which may provide better biological control (8, 31, 35). Our data indicate that K84 can be used in Spain despite the presence in soils of some isolates resistant to agrocin 84.

ACKNOWLEDGMENTS

Appreciation is expressed to L. W. Moore and C. G. Panagopoulos for kindly providing the K84 Agr and 0341 strains, to R. J. Orive and F. J. Temprano for their preparation of peat inoculants, to E. Carbonell and C. Jordà for the statistical analysis, to L. Navarro for his critical reading, and to Ana Borràs for her assistance in the translation of the manuscript.

This work was supported by grants from the Comisión Asesora de Investigación Científica y Técnica of Spain.

LITERATURE CITED

1. **Bazzi, C., U. Mazzucchi, and S. Boschieri.** 1980. Prova di lotta biologica al tumore batterico del melo in Alto Adige. Inf. Fitopatol. **6**:36.
2. **Cooksey, D. A., and L. W. Moore.** 1980. Biological control of crown gall with fungal and bacterial antagonists. Phytopathology **70**:506–509.
3. **Cooksey, D. A., and L. W. Moore.** 1982. Biological control of crown gall with an agrocin mutant of *Agrobacterium radiobacter*. Phytopathology **72**:919–921.
4. **Dhanvantari, B. N.** 1983. Etiology of grape crown gall in Ontario. Can. J. Bot. **61**:2641–2646.
5. **Du Plessis, H. J., M. J. Hatting, and H. J. J. Van Vuuren.** 1985. Biological control of crown gall in South Africa by *Agrobacterium radiobacter* strain K84. Plant Dis. **69**:302–305.
6. **El-Kady, S., and S. Süle.** 1981. Biological control of crown gall tested on bean leaves. Acta Phytopathol. Acad. Sci. Hung. **16**:307–313.
7. **Ellis, J. G., A. Kerr, M. Van Montagu, and J. Schell.** 1979. *Agrobacterium*: genetic studies on agrocin 84 production and the biological control of crown gall. Physiol. Plant Pathol. **15**:311–319.
8. **Garrett, C. M. E.** 1987. Problems of *Agrobacterium tumefaciens* in planting material and its control. Bull. OEPP/EPPO **17**:263–268.
9. **Grimm, R.** 1987. Control of crown gall in Swiss apple nurseries. Bull. OEPP/EPPO **17**:269–272.
10. **Harris, R. W.** 1931. The crown gall disease of nursery stocks. II. The relative susceptibility of apple stocks to crown gall. East Malling Res. Stn. Annu. Rep. **1928–1930**(Suppl.):140–142.
11. **Htay, K., and A. Kerr.** 1974. Biological control of crown gall: seed and root inoculation. J. Appl. Bacteriol. **37**:525–530.
12. **Kerr, A.** 1972. Biological control of crown gall: seed inoculation. J. Appl. Bacteriol. **35**:493–497.
13. **Kerr, A., and K. Htay.** 1974. Biological control of crown gall through bacteriocin production. Physiol. Plant Pathol. **4**:37–44.
14. **Kerr, A., and C. G. Panagopoulos.** 1977. Biotypes of *Agrobacterium radiobacter* var. *tumefaciens* and their biological control. Phytopathol. Z. **90**:171–179.
15. **Lelliot, R. A., and D. E. Stead.** 1987. Methods for the diagnosis of bacterial diseases of plants, p. 216. Blackwell Scientific Publications, Ltd., Oxford.
16. **Lippincott, B. B., and J. A. Lippincott.** 1969. Bacterial attachment to a specific wound site as an essential stage in tumor initiation by *Agrobacterium tumefaciens*. J. Bacteriol. **97**:620–628.
17. **Lippincott, J. A., R. Beiderbeck, and B. B. Lippincott.** 1973. Utilization of octopine and nopaline by *Agrobacterium*. J. Bacteriol. **116**:378–383.
18. **López, M. M.** 1978. Characteristics of French isolates of *Agrobacterium tumefaciens*, p. 233–237. Proceedings of the Fourth International Conference on Plant Pathogenic Bacteria, Pathologie Végétale, INRA, Angers. Gilbert-Clarey, Tours, France.
19. **López, M. M., M. T. Gorris, F. J. Temprano, and R. J. Orive.** 1987. Results of seven years of biological control of *Agrobacterium tumefaciens* in Spain. Bull. OEPP/EPPO **17**:273–280.
20. **López, M. M., M. Miró, R. Orive, F. Temprano, and M. Poli.** 1981. Biological control of crown gall on rose in Spain, p. 538–548. *In* J. C. Lozano and P. Gwin (ed.), Proceedings of the Fifth International Conference on Plant Pathogenic Bacteria. CIAT, Cali, Colombia.
21. **López, M. M., F. J. Temprano, R. Orive, M. T. Gorris, and M. Miró.** 1984. Resultados de la lucha biológica contra *Agrobacterium tumefaciens* en España, p. 321–331. Sociedad Española de Ciencias Hortícolas I Congreso Nacional, Ubeda, Almería, Spain.
22. **Moore, L. W.** 1977. Prevention of crown gall on Prunus roots by bacterial antagonists. Phytopathology **67**:139–144.
23. **Moore, L. W.** 1979. Practical use and success of *Agrobacterium radiobacter* strain 84 for crown gall control, p. 553–563. *In* B. Schippers and W. Gams (ed.), Soil borne plant pathogens. Academic Press, Inc. (London), Ltd., London.
24. **Moore, L. W., and G. Warren.** 1979. *Agrobacterium radiobacter* strain 84 and biological control of crown gall. Annu. Rev. Phytopathol. **17**:163–179.
25. **New, P. B., and A. Kerr.** 1971. A selective medium for *Agrobacterium radiobacter* biotype 2. J. Appl. Bacteriol. **34**:233–236.
26. **New, P. B., and A. Kerr.** 1972. Biological control of crown gall: field measurements and glasshouse experiments. J. Appl. Bacteriol. **35**:279–287.
27. **Panagopoulos, C. G., P. G. Psallidas, and A. S. Alivizatos.** 1979. Evidence of a breakdown in the effectiveness of biological control of crown gall, p. 570–578. *In* B. Schippers and W. Gams (ed.), Soil borne plant pathogens. Academic Press, Inc. (London), Ltd., London.
28. **Panagopoulos, C. G., P. G. Psallidas, and D. C. Stylianidis.** 1983. Current work on the effectiveness of biocontrol of crown gall, p. 67–78. International Workshop on Crown Gall, Wädenswill, Switzerland. Swiss Federal Research Station for Fruit Growing, Viticulture and Horticulture, Wädenswill, Switzerland.
29. **Schroth, M. N., and W. J. Moller.** 1976. Crown gall controlled in the field with a nonpathogenic bacterium. Plant Dis. Rep. **60**:275–278.
30. **Schroth, M. N., J. P. Thompson, and D. C. Hildebrand.** 1975. Isolation of *Agrobacterium tumefaciens-A. radiobacter* group from soil. Phytopathology **55**:645–647.
31. **Shim, J. S., S. K. Farrand, and A. Kerr.** 1987. Biological control of crown gall: construction and testing of new biocontrol agents. Phytopathology **77**:463–466.
32. **Sobiczewski, P., and S. A. Piotrowski.** 1983. Preliminary investigation on the biological control of crown gall. Fruit Sci. Rep. **10**:189–194.
33. **Spiers, A. G.** 1980. Biological control of *Agrobacterium* species *in vitro*. N.Z. J. Agric. Res. **23**:133–137.
34. **Stonier, T.** 1960. *Agrobacterium tumefaciens* Conn II. Production of an antibiotic substance. J. Bacteriol. **79**:889–898.
35. **Van Zyl, F. G. H., B. W. Strijdom, and J. L. Staphorst.** 1986. Susceptibility of *Agrobacterium tumefaciens* strains to two agrocin-producing *Agrobacterium* strains. Appl. Environ. Microbiol. **52**:234–238.
36. **Vogelsanger, J., and R. Grimm.** 1983. Collecting bacterial strains from crown galls of apple rootstocks and identification of *Agrobacterium tumefaciens*, p. 89–95. International Workshop on Crown Gall, Wädenswill, Switzerland. Swiss Federal Research Station for Fruit Growing, Viticulture and Horticulture, Wädenswill, Switzerland.