

EFFECT OF SOIL SOLARIZATION ON TOTAL *AGROBACTERIUM* SPP. POPULATION, INOCULATED *AGROBACTERIUM TUMEFACIENS*, AND ON THE DEVELOPMENT OF CROWN GALL

H. Khlaif

Department of Plant Protection, Faculty of Agriculture, University of Jordan, Amman, Jordan

SUMMARY

Two experiments were carried out in different Jordanian locations to test the effect of solarization on populations of *Agrobacterium* spp., including *Agrobacterium tumefaciens*, the crown gall agent that was inoculated in the soil, as well as on crown gall incidence. The results of these experiments showed that solarization markedly decreased the populations of three Jordanian *A. tumefaciens* isolates that were used as inoculum in the trials. Bacterial population reduction was correlated with temperature and soil type. *Agrobacterium* spp. population decreased by 99% in the first three weeks after inoculation. Lower bacterial recovery was obtained from solarized plots in the Jordan Valley (silty clay soil and temperature ranging from 39 to 51°C) than from the comparable treatments carried out in the uplands (clay soil and temperature ranging from 29 to 46°C). Solarization did not completely eliminate *Agrobacterium* spp. from the soil, but the number of GF677 and bitter almond roostock seedlings with crown gall was significantly reduced in solarized plots in the Jordan Valley and uplands (by 89 and 94%, respectively) as compared with seedlings planted on non-solarized plots.

Key words: *Agrobacterium*, crown gall, rootstocks, solarization.

INTRODUCTION

A. tumefaciens, the causal agent of crown gall disease is a soil-borne pathogen distributed worldwide, including the Mediterranean region. Over 600 plant species are reported as hosts of this disease including pome, stone, and dry fruit trees, grapevine and a wide variety of dicotyledonous plants. Nurseries and growers may suffer serious economic losses as galled plants show growth reduction, decline, and are unmarketable (DeCleene and DeLey, 1976; Lopez, 1987; Zoina and Raio, 1999; Ramon *et al.*, 2000).

Crown gall is generally characterized by tumors, but asymptomatic infection may also occur. Sensitive methods for detecting latent *A. tumefaciens* infections in plant propagating material are not yet available (Zoina and Raio, 1999).

Different control measures have been used against crown gall, among which dipping of rooted plants in a suspension of the antagonistic *Agrobacterium radiobacter* K84 (New and Kerr, 1972; Moore, 1988). However, natural *A. tumefaciens* populations that resist K84 treatment are known to exist, and K84 is not effective on infected asymptomatic plants (Alconero, 1980; Grimm and Sule, 1980; Utkhede and Smith, 1990). Furthermore, soil fumigation gives incomplete control of crown gall (Pu and Goodman, 1993) and was reported to induce an unexpected increase of disease incidence (Deep *et al.*, 1968). Chemicals and antibiotics have given incomplete crown gall control and are often phytotoxic (Grimm and Sule, 1981; Canfield *et al.*, 1992). Thus, there is a need for a safe, reliable and environmentally friendly method for elimination or reduction of *A. tumefaciens* from soil.

Soil solarization (Katan, 1980; Stapleton and Devay, 1986) proved useful for controlling plant pathogenic bacteria. For instance, it drastically reduced the population of the causal agent of tomato canker (*Clavibacter michiganensis* subsp. *michiganensis*) in plastic tunnels (Antonioni *et al.*, 1999) and, when combined with methyl bromide treatments, it also reduced the population of *Ralstonia solanacearum* (Chellemi *et al.*, 1999).

Since little is known on the effect of solarization on *A. tumefaciens* populations (Stapleton and Devay, 1982), experiments in two different locations of Jordan were conducted, to test its effects on soil populations of total *Agrobacterium* spp., of three *A. tumefaciens* strains that were inoculated in the soil, and on crown gall development.

MATERIALS AND METHODS

***Agrobacterium tumefaciens* isolates.** Three *A. tumefaciens* isolates were used to inoculate the soil, namely 251 (obtained from Dr. M. Lopez, Valencia, Spain), C 58 (obtained from Dr. X. Nesme, Lyon, France), and the Jordanian isolate 186, recovered from a peach

seedling tumor, which was identified, characterized biochemically, and pathogenetically tested according to Schaad (1988).

Field experiments. The University Research Station in the central Jordan Valley and the University of Jordan Campus in the Uplands, were selected as sites of two experiments for testing the effect of solarization on the population dynamics of *A. tumefaciens* in artificially inoculated soil, as well as on the development of crown gall on bitter almond (*Prunus amygdalus*) and GF677 (*Prunus persica* x *P. amygdalus*) seedlings used as rootstocks. In both locations the soil was tilled to a fine texture, irrigated up to field capacity, and fumigated with methyl bromide at a rate of 70-75 g/m². Each treatment consisted of one plot (10 x 5 m), replicated four times, each plot with four rows of plants one meter apart from each other and with a distance of two meters between plots. Each plot was separated from the adjacent parcels by a 50 cm deep trench, the borders of which were lined with thick plastic, prior to filling with soil to restrict possible movement of bacteria from one plot to the other.

At the beginning of August 1998, a total of 24 plots were artificially inoculated in groups of four with each of the *A. tumefaciens* strains used in this study. Inoculation was with 1·10⁸ CFU g⁻¹ of soil from a suspension of a 36 h NA culture of the bacterial isolates, and the inoculum was mixed thoroughly with the soil. Then, half of the plots were covered with clear 80 µm thick plastic film for solarization. Solarized and non-solarized plots were randomly distributed in the experimental field. The plastic cover was removed at the end of September 1998. Soil temperatures were recorded in solarized and non-solarized plots with thermometers installed at 15 cm below the soil surface.

Population dynamics of *Agrobacterium*. At each sampling date four soil samples from each treatment were collected randomly with a standard auger at a depth of 15 cm. Collection was immediately after inoculation, then at two-week intervals. Samples from all replicates of each treatment were pooled and mixed thoroughly to make a composite sample, then 10 g from each sample were taken and a 10 fold dilutions series prepared. An aliquot of 0.1 ml of the 10⁻³ dilution of each treatment was plated onto modified D1 medium (Kado and Heskett, 1970) containing (g/l) mannitol 15, NaNO₃ 5.0, LiCl 6.0, Ca(NO₃)₂·H₂O 0.02, K₂HPO₄ 2.0, MgSO₄·7H₂O 0.2, bromothymol blue 0.1, and agar 15. Four plates were inoculated with each soil sample and incubated at 25±2°C. Colonies with typical *Agrobacterium* spp. morphology were counted and the CFU/ml for each plate calculated. Thereafter, average CFU/ml of the four replicates for each treatment was calculated, and the logarithm of CFU/ml averages for each treatment plotted against time (Fig. 1 and 2). Four representative colonies showing *Agrobacterium* charac-

teristics were taken from each plate. Bacteria were identified, characterized, and their pathogenicity tested as described by Schaad (1988), for discriminating *Agrobacterium* spp. from *A. tumefaciens*.

In February 1999, all plots were planted with one-year-old healthy seedlings of GF677 and bitter almond rootstocks at 20 cm distance in two separate rows within each treatment. Drip irrigation was used throughout the experiment.

Seedlings were checked periodically for the appearance of tumors and the number of those showing tumors was recorded. Seedlings were uprooted in both experiments at the beginning of April 2000, one year after planting. The number and percentage of infected seedlings of each rootstock in each treatment was calculated, and the average weights of developed galls recorded. Isolation from five galled samples randomly collected was done on D1 media plates for *A. tumefaciens* recovery. Subculturing, identification, and pathogenicity tests were as described by Schaad (1988).

Data on the incidence and average weight of crown galls were subjected to analysis of variance and significant means differences were determined using the Duncan's multiple range test.

RESULTS

Population dynamics of *Agrobacterium*. *Agrobacterium* spp. were isolated and identified from soil samples collected at the different sampling dates. In both locations, recovered *Agrobacterium* spp. populations were much lower in solarized than in non-solarized plots. Furthermore, *Agrobacterium* populations were lower in the Jordan Valley than in the Uplands, conceivably because of the higher average soil temperature (10°C) in the first location.

Solarized plots, Jordan Valley. The average soil temperature in the solarized plots at a depth of 15 cm was 10°C degrees higher than the recorded temperatures in the non-solarized plots. This resulted in a significant reduction (*ca* 99%) in the bacterial population recovered from the solarized plots. This reduction coincided with an average soil temperature of 39 to 51°C during the period under plastic. Populations were 1·10², 3.16·10², and 3.16·10² CFU g⁻¹ soil for strains Jordan 186, C58, and 251, respectively. Thereafter, populations fluctuated according to the change in soil temperature (32 to 47°C) till the end of the experiment, ranging from 3.16·10² to 5.01·10² CFU g⁻¹ soil. The lowest population recovered was 10² CFU g⁻¹ soil (week 4), 10² CFU g⁻¹ soil (week 3), and 10 CFU g⁻¹ soil (week 5) from plots inoculated with strains Jordan 186, C58, and 251, respectively. Populations of the different isolates from similar treatments did not vary much during the remaining period (Fig. 1).

Non-solarized plots, Jordan Valley. In the non-solarized treatments populations decreased till week 3, when

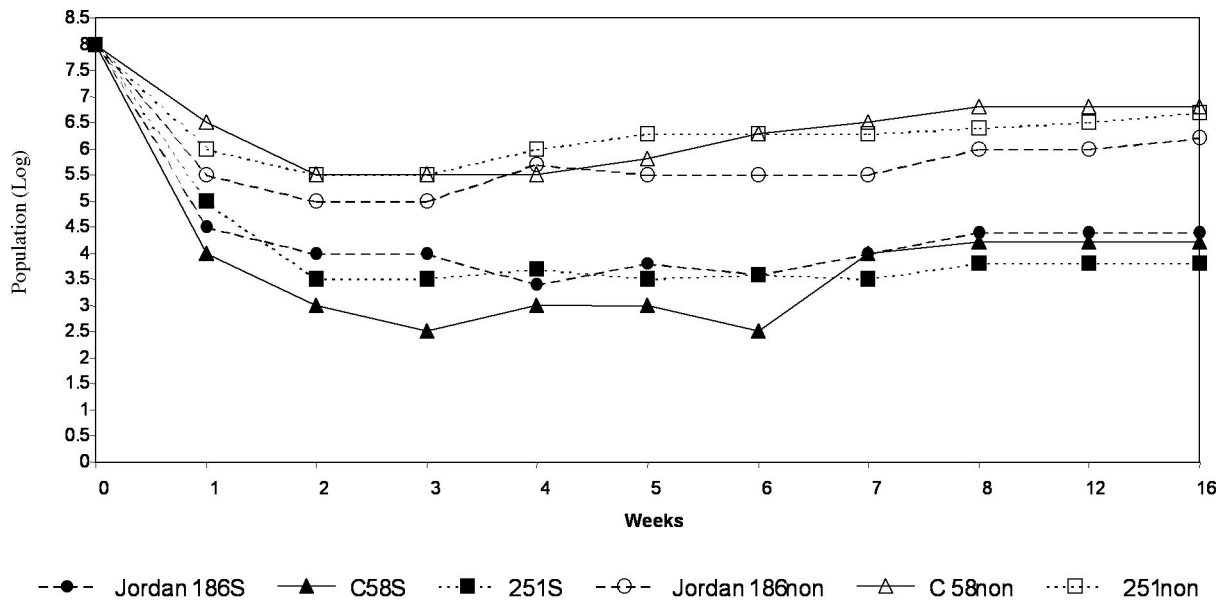


Fig. 1. Populations of *Agrobacterium* spp. in solarized and non-solarized plots in the Jordan Valley.

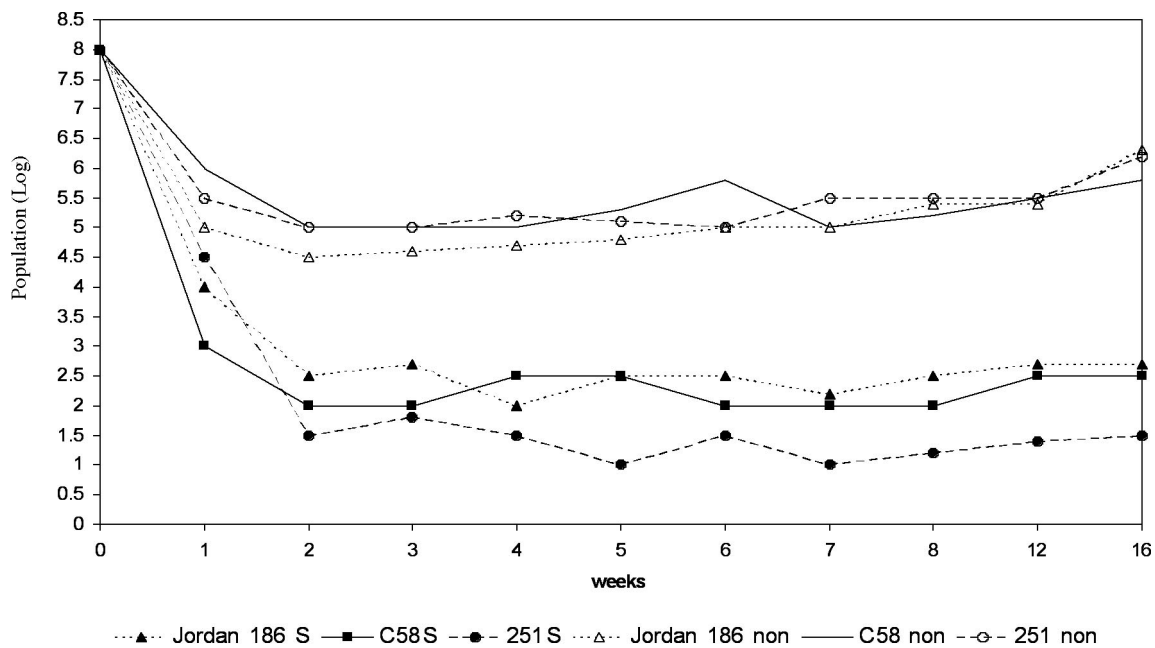


Fig. 2. Populations of *Agrobacterium* spp. in solarized and non-solarized plots in the uplands.

they were $3.16 \cdot 10^4$, $1 \cdot 10^5$, and $1 \cdot 10^5$ CFU g^{-1} for plots inoculated with strains Jordan 186, C58, and 251 respectively, coinciding with temperature of 29 up to 40°C. Thereafter, bacterial populations remained more or less stable till the end of experiment, ranging from $1 \cdot 10^5$ to $2.16 \cdot 10^5$ CFU g^{-1} soil, with temperatures ranging from 29 to 38°C (Fig. 1).

Solarized plots, Uplands. The average soil temperature in the solarized treatments at a depth of 15 cm was 5 to 7°C higher than that of the non-solarized plots, i.e. 5°C less than the recorded difference in the Jordan Valley. Nevertheless, bacterial populations

from solarized treatments that were reduced by 99% during the first three weeks, averaging $2.5 \cdot 10^3$, $3.16 \cdot 10^2$ and $3.16 \cdot 10^3$ CFU g^{-1} soil for strains Jordan 186, C58, and 251, respectively. This reduction coincided with soil temperature ranging from 27 to 46°C. Thereafter, populations fluctuated with the soil temperature (29-38°C) until they reached a stable plateau towards the end of the experiment, i.e. $1 \cdot 10^3$ to $2.5 \cdot 10^4$ CFU g^{-1} soil (Fig. 2).

Non-solarized plots, Uplands. In non-solarized treatments, bacterial populations decreased during the first three weeks when they were $1 \cdot 10^5$, $3.16 \cdot 10^5$ and $5.01 \cdot 10^5$

CFU g⁻¹ soil for plots inoculated with strains Jordan 186, C58, and 251 respectively, coinciding with a soil temperature of 27 to 34°C. Thereafter, populations fluctuated with temperature (24-31°C) until they reached a stable plateau towards the end of the experiment, i.e. 1.9·10⁶, 6.3·10⁶ and 5.01·10⁶ CFU g⁻¹ soil for strains Jordan 186, C58, and 251, respectively (Fig. 2).

Crown gall development. Pathogenic *A. tumefaciens* was isolated from tumors collected from infected rootstocks in both locations and successfully inoculated to tomato cv. Maramande seedlings. Solarization reduced significantly the percentage of infected rootstock seedlings and the average weight of crown galls in both locations, regardless to the bacterial isolate and the rootstock used.

Solarized plots, Jordan Valley. None of the almond seedlings planted in the solarized plots became infected. With GF677 infection incidence was 1.04, 2.44, and 1.8% with mean tumor weight of 11.9, 8.04, and 72.5 g for strains Jordan 186, C58, and 251, respectively.

Non-solarized plots, Jordan Valley. In the non solarized treatments planted with GF677, seedlings infection incidence was 7.01, 11.6, and 10.14%, with mean tumor weight of 68.6, 197.4 and 424.7 g for strains Jordan 186, C58, and 251, respectively. Infection incidence in almond was 4.5, 0, and 15.6, with mean tumor weight of 8.11, 0, and 54 g for strains Jordan 186, C58, and 251, respectively.

Solarized plots, Uplands. There was significant reduction in the incidence of infected seedlings planted in the solarized plots. For instance, only 1.6% of the almond seedlings planted in solarized plots inoculated with strain Jordan 186 became infected.

Non-solarized plots, Uplands. In the non-solarized treatments the incidence the infected GF677 seedlings was 0, 1.6 and 4.0%, with mean tumor weight of 0, 2.7 and 9.4 g with strains Jordan 186, C58, and 251, respectively. With almond, infection of seedlings was 5.9, 7.6, and 11.8%, with mean tumor weight of 9.13, 4.05, and 18.5 g with strains Jordan 186, C58, and 251, respectively (Table 1).

Table 1. Percentage of infected rootstock seedlings planted in solarized soil ^a.

Location	Treatment	Rootstock	Isolates	% infection	Average weight of tumors (g)	
Jordan Valley	Solarized	GF 677	Jordan 186	1.0 ef	11.9 cd	
		Almond	Jordan 186	0.0 f	0.0 d	
		GF677	C58	2.4 c	80.4 c	
		Almond	C58	0.0 f	0.0 d	
		GF677	251	1.8 e	72.5 cd	
		Almond	251	0.0 f	0.0 d	
	Non-solarized	GF677	Jordan 186	7.0 c	68.6 cd	
		Almond	Jordan 186	4.5 d	8.1 cd	
		GF677	C58	10.1 b	197.4 b	
		Almond	C58	0.0 f	0.0 d	
		GF677	251	11.6 b	424.7 a	
		Almond	251	15.6 a	54.3 cd	
		<i>L.S.D.</i>	(≤ 0.05)		1.77	79.84
		Uplands	Solarized	GF677	Jordan 186	0.0 e
Almond	Jordan 186			1.6 e	2.8 c	
GF677	C58			0.0 e	0.0 d	
Almond	C58			0.0 e	0.0 d	
GF677	251			0.0 e	0.0 d	
Almond	251			0.0 e	0.0 d	
Non-solarized	GF677		Jordan 186	0.0 e	0.0 d	
	Almond		Jordan 186	7.7 b	4.1 c	
	GF677		C58	1.6 e	9.4 b	
	Almond		C58	5.9 c	9.1 b	
	GF677		251	4.1 d	2.7 c	
	Almond		251	11.8 a	18.5 a	
	<i>L.S.D.</i>		(≤ 0.05)		1.77	1.63

^a Numbers in the same column followed with the same letter did not differ significantly in Duncan's multiple range test at P \leq 0.05

DISCUSSION

Solarization has significantly reduced *Agrobacterium* spp. populations both in the Jordan Valley and in the Uplands. Populations were substantially reduced also in non-solarized treatments, likely because of the effect of high summer temperatures in Jordan. These results are in general agreement with those by Raio *et al.* (1997).

The reduction of *Agrobacterium* spp. population was positively correlated with average temperature during the trial, for 99% reduction in *Agrobacterium* population in solarized treatments occurred in both locations during the first three weeks of the experiment. This reduction coincided with average temperature ranging from 39 to 51°C in the Jordan Valley and from 29 to 46°C in the Uplands. Solarization, however, did not completely eliminate *Agrobacterium* spp. populations. Therefore pathogenic *A. tumefaciens* may still be present in the solarized soil, and its population might build up in the next growing season, and induce appreciable disease incidence as reported in the literature (Hildebrand, 1942; Lippincott and Heberlain, 1965; Stapleton and DeVay, 1982; Raio *et al.*, 1997).

Monitoring of *Agrobacterium* spp. and especially *A. tumefaciens* in the soil is still difficult because the colonies are not always distinguishable from those of other bacteria, and highly selective media for detection of *Agrobacterium* are not yet available (Raio *et al.*, 1997) and confirmation is laborious even with molecular techniques like polymerase chain reaction.

In the present experiments, solarization resulted in 89 and 94% reduction in the incidence of crown gall-infected seedlings of both rootstocks under trial in the Jordan Valley and Uplands, respectively. These results are not in line with those by Raio *et al.* (1997), who found that solarization did not affect crown gall incidence.

Soil type was found to influence the efficiency of solarization that, in turn, affected the reduction in crown gall incidence in both locations. The silty clay soil of the Jordan Valley was found to be more favorable to *Agrobacterium* survival than the heavy soil of the Uplands, as the number of infected rootstock seedlings was lower in the latter location. These results are in general agreement with those by Raio *et al.* (1997) who reported that *A. tumefaciens* survived longer and was less affected by solarization in heavy clay loam soil.

Summarizing, solarization is a simple and cheap method for controlling *A. tumefaciens* which, as shown by our results, looks very promising also under Jordanian conditions and can successfully be used in an integrated control strategy for crown gall disease.

ACKNOWLEDGEMENTS

This work was supported by EU contract ERBIC 18CT970198. "Integrated control of crown gall in

Mediterranean countries". The author wishes to thank Prof. Naim Sharaf and Prof. B. Abu Irmaileh for reviewing this manuscript.

REFERENCES

- Alcornero R., 1980. Crown gall of peaches from Maryland, South Carolina, and Tennessee and problems with biological control. *Plant Disease* **64**: 835-838.
- Antoniou P.P., Tjamos E.C., Panagopoulos C.G., 1995. Use of soil solarization for controlling bacterial canker of tomato in plastic houses in Greece. *Plant Pathology* **44**: 438-447.
- Canfield M.L., Pereira C., Moore L.W., 1992. Control of crown gall in apple (*Malus*) rootstocks using Copac E and Terramycin. *Phytopathology* **82**: 1153.
- Chellemi D.O., Olson S.M., Mitchell D.J., 1994. Effects of soil solarization and fumigation on survival of soil borne pathogens of tomato in Northern Florida. *Plant Disease* **78**: 1167-1172.
- DeCleene M., Deley J., 1976. The host range of crown gall. *Botanical Review* **42**: 390-466.
- Deep I.W., McNeilan R.A., Macswan I.C., 1968. Soil fumigants tested for control of crown gall. *Plant Disease Reporter* **52**: 102-105.
- Grimm R., Sule S., 1981. Control of crown gall (*Agrobacterium tumefaciens* Smith & Townsend) in nurseries. In: Lozano JC, Gwin P, (eds). *Proceedings of the 5th International Conference on Plant Pathogenic Bacteria, Cali, Colombia, 531-537*.
- Hildebrand E.M., 1942. A micrurgical study of crown gall infection in tomato. *Journal of Agricultural Research* **65**: 45-59.
- Kado C.I., Heskett M.C., 1970. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. *Phytopathology* **60**: 969-976.
- Katan J., 1980. Solar pasteurization of soil for disease control: status and prospects. *Plant Disease* **64**: 450-454.
- Lippincott J.A., Heberlain G.I., 1965. The quantitative determination of infectivity of *Agrobacterium tumefaciens*. *American Journal of Botany* **52**: 856-863.
- Lopez M., 1987. Results of seven years of biological control of *Agrobacterium tumefaciens* in Spain. *Bulletin OEPP/EPPPO Bulletin* **17**: 273-279.
- Moore L.W., 1988. Use of *Agrobacterium radiobacter* in agriculture ecosystem. *Microbiological Sciences* **5**: 92-95.
- New P.B., Kerr A., 1972. Biological control of crown gall: field measurements and glass house experiments. *Journal of Applied Bacteriology* **35**: 279-287.
- Pu X.A., Goodman R.N., 1993. Effects of fumigation and biological control of infection of indexed crown gall free grape plants. *American Journal of Ecology and Viticulture* **44**: 244-249.
- Raio A., Zoina A., Moore L.W., 1997. The effect of solar heating on natural and inoculated agrobacteria. *Plant Pathology* **46**: 320-328.

- Ramon P., Vicedo B., Lopez M., 2000. Use of the genetically engineered *Agrobacterium* strain K1026 for biological control of crown gall. *European Journal of Plant Pathology* **9**: 801-810.
- Schaad N.W., 1988. Laboratory Guide of Plant Pathogenic Bacteria. 2nd Ed. APS Press, St. Paul, Minnesota, USA.
- Stapleton J.J., DeVay J.E., 1982. Effect of soil solarization on populations of selected soil borne microorganisms and growth of deciduous fruit tree seedlings. *Phytopathology* **72**: 323-326.
- Stapleton J.J., DeVay J.E., 1986. Soil solarization: a non-chemical approach for management of plant pathogens and pests. *Crop Protection* **5**: 190-198.
- Utkhede R.S., Smith E.M., 1990. Effects of fumigants and *Agrobacterium radiobacter* in the environment. *Applied and Environmental Microbiology* **59**: 2112-2120.
- Zoina A., Raio A., 1999. Susceptibility of some peach rootstocks to crown gall. *Journal of Plant Pathology* **81**: 181-187.

Received 2 September 2002

Accepted 15 April 2003