Effect of Inocula Delivery Systems on Rhizobacterial Colonization of Underground Organs of Potato

J. B. Bahme, M. N. Schroth, S. D. Van Gundy, A. R. Weinhold, and D. M. Tolentino

Graduate research assistant and professor, Department of Plant Pathology, University of California, Berkeley 94720; professor, Department of Nematology, University of California, Riverside 92521; professor, Department of Plant Pathology, University of California, Berkeley 94720; research plant pathologist, Instituto Guido Donegani, Novara, Italy. Present address of the first author: AgBioChem, Inc., 10795 Bryne Avenue, Los Molinos, CA 96055.

We thank Marc Monson for technical assistance, Frank Murillo for preparation of graphics, and George Papivizas for providing *Trichoderma viride* strain T-1-R9.

This research was supported in part by the California Potato Research Advisory Board.

Accepted for publication 19 October 1987 (submitted for electronic processing).

ABSTRACT

Bahme, J. B., Schroth, M. N., Van Gundy, S. D, Weinhold, A. R., and Tolentino, D. M. 1988. Effect of inocula delivery systems on rhizobacterial colonization of underground organs of potato. Phytopathology 78:534-542.

The effect of delivering *Pseudomonas fluorescens* strain A1-B to soil by incorporation of bacteria-impregnated granules and injection through a low-pressure drip irrigation system on the resultant colonization patterns was determined at all stages of plant development on below-ground parts of field-grown potato in two different soil types. Strain A1-B was released from granules during irrigations and persisted at mean populations of approximately 10^2 cfu g^{-1} in nonrhizosphere soil beneath the zone of granule incorporation. Populations of strain A1-B in the granules declined from an initial density of 10^9 cfu g^{-1} to 10^5 by harvest time. Periodic application of strain A1-B through the drip irrigation system resulted in passive distribution of the bacteria throughout the top 30 cm of beds and established season-long mean population sizes of approximately 10^5 cfu g^{-1} in nonrhizosphere soil. Soil population densities of drip-introduced strain A1-B gradually increased during the season in silty clay loam but decreased in sandy loam. Mean rhizosphere population sizes (log cfu cm^{-1}) of strain A1-B on root segments 0–8, 8–16, and 24–32 cm distal to points of root origin in granule plots were 3.4, 4.4, and 0.7, respectively, in sandy loam, and 3.1, 2.2, and 1.4 for the same respective segments in silty clay loam. Rhizosphere populations on these respective root segments in drip plots were 4.1, 3.9, and 1.6 in sandy loam and 3.7, 2.8, and 2.2 in silty clay loam. The population densities and uniformity of colonization were much greater with granule and drip delivery systems than with seed-piece inoculation. Mean root tip population sizes (log cfu per tip) of strain A1-B on roots of 8 and 16 cm in total length were 3.3 and 0.7, respectively, in granule plots and 3.8 and 1.2 on roots of the same respective lengths in drip plots. Introduction of strain A1-B to soil by granule and drip delivery systems also resulted in large, relatively uniform populations of the bacterium on progeny tubers. Populations of strain A1-B in nonrhizosphere soil in drip plots accounted for 1 and 630% of the total detectable aerobic bacteria and native fluorescent pseudomonads, respectively. Rhizosphere populations of granule-introduced and drip-introduced strain A1-B on root segments adjacent to seed pieces constituted 0.22 and 0.17%, respectively, of the total detectable aerobic bacteria of these segments and 35 and 27% of the fluorescent pseudomonads for the same respective systems of delivery. Population densities of drip-applied strain A1-B were approximately 1 log unit greater in nonrhizosphere soil and on roots and progeny tubers in plots preplant-fumigated with metham sodium. Drip-irrigation injection of conidia of *Trichoderma viride* resulted in distribution of the fungus throughout beds and established season-long mean nonrhizosphere soil population sizes of log 3.5 cfu g^{-1} in silty clay loam and log 1.0 cfu g^{-1} in sandy loam.

The composition of the rhizosphere microflora has generally been considered to be relatively resistant to destabilization because of the buffering effects of the biotic and abiotic environments. However, recent research has shown that the rhizosphere microflora can be altered easily by inoculating plant parts with specific rhizobacteria (13,18,23). The amount of alteration presumably depends on the nature of the introduced strain and its capacity to effectively colonize and persist on root systems for extended time periods. The size of the inoculum (15) and how it is delivered to potential colonization sites directly affect subsequent population dynamics. For example, field and greenhouse studies suggest that population densities on roots decline markedly with distance from the source of original inoculum (1,15,20). Consequently, seed-inoculated bacterial antagonists have been more effective in suppressing fungi that infect seedlings (8,9,21) than those that attack growing root tips and plant parts distal to the site of inoculation and zone of greatest colonization (22).

The population sizes and distribution of seed-inoculated rhizobacteria on roots are highly variable, even on plant parts near the source of inoculum (1,15). This variability also occurs among root systems of individual plants (14). To fully realize the disease-reducing potential of antagonistic rhizobacteria, methods are needed to increase the distribution and density of the antagonists on plant parts to attain a more uniform and durable alteration of the rhizosphere. This could include the development of new systems for site delivery of rhizobacteria to plant parts and soil. A reservoir of inoculum ideally should be situated in soil space that will be explored by the growing roots because some key constraints curtail colonization of the growing root tips by seed-introduced rhizobacteria. These constraints are the physical removal of root apex bacteria as the advancing root tip displaces and realigns soil particles in a tight sheath about the root (5), adsorption of bacteria to soil colloids (16), competition from native bacteria encountered in the soil, and rhizobacterial generation times too slow to keep pace with plant growth.

This study reports the development of two new delivery systems for applying rhizobacteria to underground organs of potato and the effect of the systems on the resultant colonization patterns of the introduced microorganisms. As an additional point of interest, the below-ground distribution patterns of conidia of a benomyl-tolerant strain of *Trichoderma viride* were investigated.

MATERIALS AND METHODS

Bacterial and fungal strains. *Pseudomonas fluorescens* strain A1-B was selected for rhizosphere investigations because of past characterization studies (1,13). A rifampicin-resistant mutant of strain A1-B was used in all experiments. A benoymyl-tolerant *Trichoderma viride* Pers. ex S.F. Gray strain, T-1-R9 (17), was used to evaluate below-ground distribution of fungal conidia by drip-irrigation injection.

Formulation, storage life, and releasing properties of bacterial granules. Uniform, 2-mm-diameter perlite granules (Perlite Processing, Inc., Santa Fe Springs, CA) were impregnated with

strain A1-B by saturating the granules for 10 min with a suspension of 5×10^9 cfu ml^{-1} made from cultures growing on King's Medium B (KB) (10) for 48 hr at 27 C. The suspension was made with sterile 0.1 M phosphate buffer (pH 7.0) and then blended with an equal volume of a sterile 1.0% methyl cellulose suspension (Methocel 65 HG, Dow Chemical Co., Midland, MI) until a uniform bacterial slurry was obtained. The saturated granules were spread on metal trays, slowly dried at 15–17 C for 48 hr, and then bulked and stored in plastic bags at 4 C until used.

Survival characteristics of strain A1-B in granules stored at 4 C and room temperature were determined by monitoring population densities of the bacterium weekly for the first month after formulation and at monthly intervals for four more months. One-gram samples were suspended in 10 ml of sterile phosphate buffer and agitated three times for 2-min intervals with a vortex mixer followed by 10 min of nonagitation. Serial 10-fold dilutions then were made to 10^{-5}. One-tenth-milliliter aliquots of the appropriate dilutions were each plated onto KB amended with 100 μg ml^{-1} rifampicin (Sigma Chemical Company, St. Louis, MO), 100 μg ml^{-1} cycloheximide (Sigma Chemical Company), and 50 μg ml^{-1} benomyl (Benlate). Colony counts were made after 48-hr incubation at 27 C, and populations of A1-B per gram granules were determined.

The bacteria-releasing properties of granules impregnated with strain A1-B were measured in water and soil in the laboratory. For the water assay, 1-g samples of granules of known bacterial density were placed in each of 15 glass test tubes. Then a tightly fitting circle of 0.5-mm^2 mesh nylon screen was inserted down into each tube and seated over the top of the granules to prevent flotation of the buoyant granules. Ten milliliters of phosphate buffer was gently added to each tube, and the tubes were held vertical and motionless until further assay. At 30, 60, and 120 min, the phosphate buffer was decanted from each of five tubes and individually diluted to 10^{-5}. One-tenth milliliter aliquots of undiluted suspension and the dilutions were plated on KB amended with the antibiotics (KB-rif), and the plates were incubated at 27 C for 48 hr. The number of cells released from the granules over time was calculated on the basis of colony counts.

For the soil assay, 3-g samples of granules of known bacterial density were mixed into each of three 300-cm^2 portions of field soil (Osborn silty clay loam, pH 7) and placed into Styrofoam cylinders (12.7-cm diameter) on top of a 4-cm-thick layer of field soil. A 0.5-mm^2 mesh nylon screen covered the bottom of the cylinders. Each cylinder was then inserted approximately 10 cm deep into a slightly larger cylinder containing 800 cm^2 of granule-free soil and firmly seated so that the nylon mesh was fully integrated with the upper surface of the soil below to create a continuous soil column. Soil in the cylinders was packed to a constant bulk density. To simulate downward water movement through soil as occurs during irrigation in the field, sterile water was slowly dripped onto the top surface of the granule-incorporated soil until free water escaped out the bottom of the system (approximately 8 hr). Granules were then recovered from soil in the top cylinders and assayed for populations of strain A1-B as described above. Granule-free soil contained in the lower cylinders was assayed for populations of strain A1-B released from granules and carried down the soil column in water. Twenty grams of soil was removed from each of the three lower cylinders, separately agitated in 50-ml phosphate buffer at 200 rpm for 15 min, diluted to 10^{-5}, plated on KB-rif, and incubated as described above. Colony counts were made, and population densities of strain A1-B per gram granule and soil were determined. The upper cylinders containing granule-incorporated soil were placed in a 15 C incubator and allowed to slowly dry (2 wk). Populations of strain A1-B in granules were assayed again, the cylinders seated as before over fresh field soil, and the experiment was repeated to simulate a second irrigation.

Experimental design of colonization studies. Experiments were conducted at the University of California South Coast Field Station (South Coast) near Tustin, CA, and at the Tulelake Field Station, Tulelake, CA. Soil type at South Coast was a San Emigdio sandy loam with negligible organic matter. The Tulelake soil was an Osborn silty clay loam with 10% organic matter (peat) in the surface horizon. Soil pH at both sites was approximately 7.0. Investigations with strain A1-B were done in separate drip-irrigated and sprinkler-irrigated experiments at each of the two sites in 1985. Strain T-1-R9 was studied in drip plots at Tulelake (1985) and South Coast (1986). The plots were arranged in random blocks with one-row plots, each 3.04 m long. Each treatment was replicated five times. Beds were 55 cm wide and on 102-cm centers. Potato cultivar White Rose was used at South Coast and cultivar Russet Burbank at Tulelake. Suberized, uniform seed pieces (approximately 56 g) were planted at a depth of 16 cm and at a spacing of 20 cm. Colonization studies were done with potato except in T-1-R9 plots at South Coast (1986), where tomato variety UC-82 was used.

Granule application. The bacterial granules were mechanically incorporated just before planting into a zone of soil approximately 20 cm wide and approximately 20 cm deep along the centers of preformed beds in sprinkler-irrigated plots at a rate of 15 g per 30 linear centimeters of row. This resulted in an overall initial inoculum load of approximately 2.5×10^6 cfu strain A1-B per cubic centimeter of soil in the granule-incorporated zone. Seed pieces were then planted into this treated soil.

Low-pressure drip irrigation delivery. Inoculum for injection through the low-pressure drip irrigation system applicator (19) was prepared just before treatment by suspending strain A1-B growth from 20 175 × 25 mm KB petri plate cultures (48 hr at 27 C) in 1-L of phosphate buffer. This method gave consistent inocula of approximately 5×10^9 cfu ml^{-1}. For delivery to soil, the bacterial suspension was added to the appropriate treatment cannister from which it was injected into the drip irrigation water stream over a period of 1 hr. This water was uniformly applied to each of five plots located in separate random blocks. The drip tubes ran the length of the plots and were buried 2 cm beneath the soil surface. Eleven in-line emitters per plot spaced at approximately 30-cm intervals delivered irrigation water containing an average of approximately 4.0×10^7 cfu ml^{-1} to the soil at a rate of 2 L per emitter per hour. Drip applications of strain A1-B were made immediately after planting at both sites and then at 21, 41, and 91 days postplant at South Coast and at 28, 63, and 96 days after planting at Tulelake. Bacterial suspensions were always injected during the final hour of irrigation runs that varied in duration from 4 to 8 hr, depending on crop water requirements.

At South Coast, strain A1-B also was introduced by drip irrigation delivery to soil of plots treated preplant with metham sodium. Applications of strain A1-B were made on the same treatment dates and according to the same procedures described for South Coast drip plots. The metham sodium was applied 2 wk before planting at a rate of 33 ml active ingredient per square meter of soil and in enough water to move the chemical to a depth of approximately 15 cm.

Conidial suspensions of approximately 3.0×10^7 conidia ml^{-1} of *T. viride* were prepared immediately before application by removing conidia from potato dextrose agar (PDA) cultures 20 175 × 25 mm petri plates) of strain T-1-R9 and thoroughly mixing in 1 L of phosphate buffer amended with two drops of tergitol NP-10 (Sigma Chemical Company). T-1-R9 cultures were incubated under continuous illumination (1,000 lux) for 1 wk at room temperature (approximately 25 C). This inoculum was introduced into the low-pressure drip irrigation system applicator and delivered to the soil as described for rhizobacterial strain A1-B. Treatments were made on the same schedule as strain A1-B at Tulelake, and at South Coast they were made at planting and at 26 and 62 days postplant (1986).

Seed-piece inoculation. Dry seed-piece inoculum was prepared with a final bacterial density of approximately 5×10^8 cfu g^{-1} (12). Seed pieces were dusted with inoculum immediately before planting, resulting in average populations of 10^8 cfu per seed piece.

Granule and seed-piece inoculum levels. Populations of strain A1-B in soil-incorporated granules and on planted, treated seed pieces were monitored at planting at both locations, and again at 21, 41, 91, and 119 days postplant at South Coast and at 28, 63, 96, and 138 days after planting at Tulelake. Individual seed pieces or soil samples containing granules were placed in plastic bags,

transported to the laboratory in an iced chest, and processed within 24 hr of sampling. One-gram samples of granules were retrieved with forceps from each of four soil samples and assayed as previously described for granules. Five seed pieces (one per plot) were individually shaken (200 rpm) in 100 ml of phosphate buffer for 15 min. Then serial dilutions were made to 10^{-3}, and 0.1-ml aliquots were plated on KB-rif as described above. Colony counts were made after 48-hr incubation at 27 C, and population sizes of strain A1-B per gram granule and per seed piece were determined.

Distribution of strains A1-B and T-1-R9 in nonrhizosphere soil. Strain A1-B was assayed in nonrhizosphere soil at various depths to determine the extent of downward movement and distribution from granules, drip emitters, and inoculated seed pieces. Samplings were made before and after irrigations to determine the role of irrigation water in dispersal. Ten-gram soil samples were collected from the soil profile at points 5 cm and 15 cm below the granule-incorporated zone and at the same points beneath treated seed pieces. Soil samples in drip plots were taken at 15 and 30 cm directly below drip emitters and at a depth of 30 cm midway between adjacent emitters. Samples were transported to the laboratory in iced chests and stored at 4 C until processed (<48 hr). These samples were individually suspended in 50 ml of phosphate buffer and agitated as described for seed pieces. Serial 10-fold dilutions were made to 10^{-4}, plated on KB-rif, and incubated as described above. Colony counts were made, and population densities of strain A1-B per gram soil at each depth were determined. Extreme care was used to collect samples free from rhizosphere soil. Ten sets of samples, two sets from each of the five plots, were taken for each treatment at each sampling date. Samples were collected at 21, 41, 91, and 119 days after planting at South Coast and at 28, 63, 96, and 138 days postplant at Tulelake. The first, second, and third samplings of drip plots at both sites were done just prior to the second, third, and fourth applications of strain A1-B.

Distribution of *T. viride* strain T-1-R9 in soil beneath the drip emitters at Tulelake and South Coast was evaluated as described for drip-applied strain A1-B, except that soil dilutions were plated on PDA amended after autoclaving with sterile solutions of streptomycin (100 μg ml^{-1}), penicillin G (50 μg ml^{-1}), polymyxin B sulfate (50 μg ml^{-1})(Sigma Chemical Company), benomyl (500 μg ml^{-1}), and 0.2% tergitol NP-10. Plates were incubated as described above for T-1-R9 cultures until discreet, characteristic conidia-bearing colonies could be quantified, after which numbers of T-1-R9 propagules per gram soil were determined. Soil samples at Tulelake were taken at the same dates as for strain A1-B, while sampling at South Coast (1986) was done 24 hr after the drip application at planting, at 2, 9, and 15 days postplant, and just before and 24 hr after treatments at 26 and 63 days.

Colonization of roots, underground portions of shoots, and progeny tubers. The colonization patterns of strain A1-B on potato after introduction of the bacterium to soil by soil-incorporated granules, drip irrigation injection, and seed-piece inoculation were determined by monitoring populations on developing roots, underground portions of shoots, and progeny tubers. Root population sizes and distributions were assessed at 21, 41, and 91 days after planting at South Coast and at 28, 63, and 96 days postplant at Tulelake. On each of these dates, drip plots were sampled before injection of strain A1-B. At the first root sampling date at both sites, individual roots (arising adjacent to seed pieces) were excised at their point of attachment to stems, shaken to remove excess soil, and the initial 0–8-cm segments and contiguous 8–16-cm segments of root were assayed separately. Single segments were cut up, placed into disposable 1.5-ml centrifuge tubes containing 1 ml ofphosphate buffer, and agitated for 10 min with an Eppendorf Mixer No. 5432 (Brinkmann Instruments, Inc., Westbury, NY). Serial dilutions were made to 10^{-2}, and 0.1-ml aliquots of the washing mix and dilutions were each plated on KB-rif. The plates were incubated as described above, colonies were counted, and population sizes of strain A1-B per centimeter root were determined. At the second and third root sampling dates, the proximal 0–8-cm segments and distal 24–32-cm segments of individual roots were assayed in the same manner. At each sampling date, those roots exhibiting the most advanced stages of growth and of approximately equal diameter and length were selected. Ten roots (two per plot), each from a separate plant, were collected for each delivery system at each sampling date.

The advancing tips of roots with points of origin on stems near to seed pieces were monitored for populations of strain A1-B at 21 and 41 days postplant at South Coast and 28 and 63 days after planting at Tulelake. The apical 0.5-cm tips were excised from roots measuring 8 and 16 cm in total length at the first and second sampling dates, respectively. Ten tips (two per plot), each from a different plant, were individually washed and plated as described above for each delivery system at each sampling date.

Underground portions of shoots extending above seed pieces were assayed for populations of strain A1-B at 21 and 28 days postplant at South Coast and Tulelake, respectively. Ten shoots per delivery system (two per plot), each from a different plant, were excised from seed pieces and the proximal 0–8-cm segment was assayed. Individual segments were placed in 5 ml of phosphate buffer, agitated as described above for granules, and plated on KB-rif.

Populations of strain A1-B on developing and mature progeny tubers were monitored by peeling the individual tubers and washing the peels in 50–100 ml of phosphate buffer, making three 10-fold serial dilutions, and plating each on KB-rif. Peelings for each tuber were dried (50 C for 48 hr), and populations of strain A1-B per gram dry weight of peel were determined. Tubers arising from stolons with points of attachment to stems near to and those attached >8 cm distal to the seed pieces were assayed separately. Ten tubers (two per plot) of approximately equal size, each from a different plant, were assayed at each sampling date for each position and each delivery system. Progeny tubers were sampled at 41, 91, and 119 days after planting at South Coast and at 63, 96, and 138 days postplant at Tulelake.

Populations of *T. viride* strain T-1-R9 on roots, underground portions of shoots, and progeny tubers. Populations of strain T-1-R9 were quantified on potato roots, underground portions of shoots, and progeny tubers at Tulelake in 1985, and on tomato roots at South Coast in 1986. At Tulelake, individual roots arising adjacent to seed pieces were excised and shaken to remove excess soil, and segments 0–8, 8–16, and 24–32 cm distal to points of attachment to stems were assayed at 28, 63, and 96 days after planting, respectively. Samples were taken before drip irrigation delivery of T-1-R9 on these dates. Ten roots (two per plot), each from a different plant, were sampled at each sampling date. Individual segments were processed as described for root segments and plated on PDA amended with benomyl and the antibiotics as previously indicated (PDA-benomyl). Shoot segments were assayed for T-1-R9 at 28 days postplant as described for shoots, except that washing mix and dilutions to 10^{-1} were plated on PDA-benomyl. Populations of T-1-R9 on progeny tubers were monitored at 63, 96, and 138 days after planting on tubers arising from stolons with points of attachment near to seed pieces. Ten tubers (two per plot) were assayed at each date, and samples were processsed as described for tubers and plated on PDA-benomyl. At South Coast in 1986, populations of T-1-R9 on tomato root segments 0–8 and 8–16 cm distal from points of root attachment to crowns were assayed at 26 and 62 days after planting, respectively, as described for potato roots.

Population sizes of total aerobic and native fluorescent pseudomonad bacteria. Populations of total aerobic bacteria and indigenous fluorescent pseudomonads were assessed in soil and on potato roots and tubers of nontreated plots at South Coast and Tulelake. Ten 10-g soil samples (two per plot) were collected at points 15 cm below the soil surface from untreated control plots at the same dates given above for sampling of strain A1-B plots. Individual samples were placed in 50-ml phosphate buffer and agitated as described for seed pieces. Serial 10-fold dilutions were made to 10^{-6}, and 0.1-ml aliquots were plated onto KB and S1 medium (7) to estimate population sizes of total aerobic bacteria and fluorescent pseudomonads, respectively. Colonies of fluorescent bacteria were enumerated under ultraviolet irradiation (360 nm). Preliminary studies indicated that recovery rates of

aerobic bacteria in the South Coast and Tulelake soils were slightly greater with KB than with general media such as 20% tryptic soy agar.

Population sizes of these bacteria were also estimated on the initial 0–8-cm segments of roots arising adjacent to seed pieces at South Coast and Tulelake at 21 and 28 days postplant, respectively. Ten root segments (two per plot), each from a different plant, were individually washed in 1 ml of phosphate buffer, serial dilutions were made to 10^{-6}, and 0.1-ml aliquots were plated on KB and S1. Similarly, populations on each of 10 progeny tubers positioned near seed pieces were estimated at 91 and 119 (time of harvest) days postplant at South Coast and at 63 and 138 (harvest) days at Tulelake. Tuber samples were processed as described for tubers, dilutions were made to 10^{-6}, and aliquots were plated on the two media.

Data analysis. Populations of strain A1-B, strain T-1-R9, total aerobic bacteria, and fluorescent pseudomonads were estimated from dilution plates with counts ranging from 1 to 300 colonies per plate. The contribution of each plate count to the calculated mean was proportional to its dilution from the original washing mix. Bacterial populations were expressed as colony-forming units per centimeter of root or shoot, per root tip, per gram soil or periderm, and as the logarithms (base 10) of these values. All data sets were analyzed for normality with the univariate procedure (Statistical Analysis Systems release 79.6, SAS Institute, Inc., Cary, NC). Because all populations conformed more closely to a lognormal rather than a normal probability distribution, the log transformation was applied to raw data values prior to statistical analysis. Significance tests among population means for drip delivery, granule, and seed-piece inoculation plots are qualified in that drip-irrigated investigations were done in separate, but adjacent, experiments to those irrigated by sprinkler.

RESULTS

Populations of strain A1-B in granules and on inoculated seed pieces. Strain A1-B persisted in high numbers in granules stored at 4 C and at room temperature (24 C). Mean population sizes (cfu g^{-1}) in granules decreased from 1.2×10^9 at time of formulation to 2.2×10^8 and 1.6×10^7 after 146 days for granules stored at 4 C and room temperature, respectively. Population sizes of strain A1-B released from granules held motionless in sterile phosphate buffer were 2.3×10^7, 2.8×10^7, and 5.3×10^7 cfu after 30, 60, and 120 min, respectively. These numbers represented 1.9, 2.3, and 4.4%, respectively, of the total, initial granule inoculum load of 1.2×10^9 cfu g^{-1}.

Strain A1-B was released from granules by the action of the downward percolation of water through field soil in the laboratory and was detected at mean population sizes of 3.6×10^5 cfu g^{-1} in soil beneath the granule-incorporated zone (below the nylon screen) after the first application of water. Mean population densities of strain A1-B in granules recovered from soil after the first watering cycle (1.0×10^9 cfu g^{-1}) were slightly less than the initial population densities at the start of the experiment (1.2×10^9 cfu g^{-1}). After a 2-wk incubation at 15 C, average populations of the bacterium in granules recovered from soil that had been irrigated one time had declined by approximately 0.5 log units to 4.7×10^8 cfu g^{-1}. Following the second watering cycle, strain A1-B was detected in fresh field soil beneath the zone of granule incorporation at mean population sizes of 2.4×10^5 cfu g^{-1}. Granules recovered from twice-irrigated soil had population densities of strain A1-B of 1.8×10^8 cfu g^{-1}.

Monitoring populations of strain A1-B in granules recovered from soil in field plots showed that population densities had declined by approximately 2.5 log units 40–60 days postplant (Fig. 1). Relatively high numbers of bacteria were associated with the granules during the entire growing season with average residual population densities of approximately 4×10^5 cfu g^{-1} granules at harvest at both sites.

Strain A1-B persisted at relatively high population sizes on seed pieces at South Coast and Tulelake throughout the growing seasons. Seed-piece populations declined from average initial sizes of approximately 10^8 cfu at planting (both sites) to 8.6×10^5 cfu per seed piece at South Coast and 4.4×10^5 cfu per seed piece at Tulelake, by harvest. Most seed pieces (approximately 90%) at South Coast remained sound throughout the season, while most at Tulelake gradually decayed, leaving only partially intact periderms to sample at harvest time.

Distribution of strain A1-B in nonrhizosphere soil. Delivery of strain A1-B to soil by incorporation of bacteria-impregnated granules resulted in establishment of season-long populations of the bacterium in nonrhizosphere soil spanning the length of the beds beneath the zone of granule incorporation. Strain A1-B was dispersed downward in nonrhizosphere soil with the first sprinkler irrigation at South Coast and Tulelake to a depth of 15 cm beneath the lower boundary of the zone of granule incorporation. Soil population sizes within this range in granule plots were similar at both sites. Average populations at soil depths of 5 and 15 cm beneath the granule zone persisted at 4.5×10^2 and 23 cfu g^{-1} soil, respectively, 21–96 days after planting. After this time, mean values dropped to 20 and 11 cfu g^{-1} by harvest, for the same respective depths.

Periodic delivery of strain A1-B to soil in drip irrigation water resulted in passive distribution of the bacteria throughout the top 30 cm of the beds and established season-long mean population sizes of approximately 10^5 cfu g^{-1} in nonrhizosphere soil within this zone (Fig. 2). Average population sizes of strain A1-B at both sites were approximately 1 log unit greater at a soil depth of 15 cm than at 30 cm when measured in a zone directly beneath the emitters, and about half a log unit greater at this latter zone than at a soil depth of 30 cm midway (15 cm) between adjacent emitters. Monitoring populations of strain A1-B in soil at intervals just before the second, third, and fourth drip applications and at harvest showed that population sizes in the sandy loam soil at South Coast were approximately 0.5 log units greater at the first sampling date than values for corresponding soil zones at Tulelake. However, populations at South Coast gradually declined during the remainder of the season, in spite of repeated reintroduction of strain A1-B. In contrast, populations of strain A1-B in the silty clay loam at Tulelake generally increased during the season and were approximately 1 log unit greater at all three sampling positions than at corresponding soil zones at South Coast by harvest time.

Preplant treatment of soil with metham sodium resulted in significantly increased soil populations of drip-introduced strain A1-B compared to nonfumigated A1-B drip plots. Soil population densities of strain A1-B at a depth of 15 cm beneath emitters were greater ($P = 0.05$) in metham-sodium-treated plots at South Coast by approximately 0.8 log units at 21, 41, and 119 days after planting than in previously described nonfumigated plots.

Strain A1-B was distributed by sprinkler irrigation water in nonrhizosphere soil to a detectable depth of approximately 5 cm directly beneath inoculated seed pieces at both field sites. Populations of strain A1-B at this depth at South Coast and

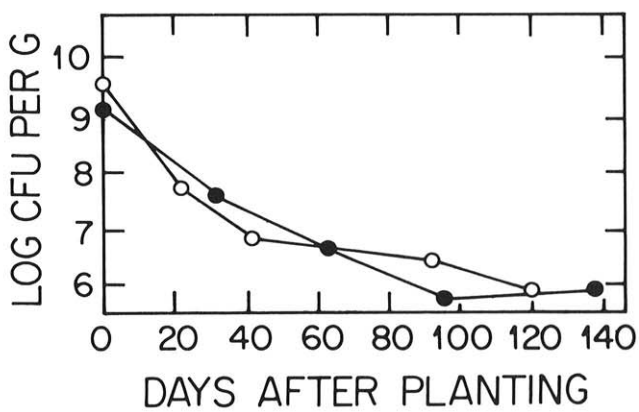

Fig. 1. Population densities of *Pseudomonas fluorescens* strain A1-B in bacteria-impregnated granules recovered from soil during the season at South Coast (o) and Tulelake (•), CA.

Tulelake were similar in size and persisted at average densities of 2.1×10^2 cfu g^{-1} from 21 to 96 days postplant, then declined to 36 cfu g^{-1} by harvest.

Rhizosphere populations of strain A1-B. Rhizosphere populations of strain A1-B were substantially greater in drip injection and bacterial granule plots than in plots where strain A1-B was applied to seed pieces. Mean population sizes on the initial 0–8-cm segments of roots arising adjacent to seed pieces were significantly greater ($P = 0.05$) with the drip and granule delivery systems than with seed-piece inoculation at all sampling dates at South Coast (Fig. 3). This also occurred at Tulelake except at the first sampling date, when population means of granule plots were not significantly greater than those of seed piece plots. Population means on these root segments in drip plots were greater than those from granule plots at both sites, but this was only statistically significant at the final sampling dates. Root population sizes on the initial 0–8-cm segments in drip, granule, and seed-piece inoculation plots at the first sampling date were 0.7–1.5 log units greater in the sandy loam soil at South Coast than in the silty clay loam at Tulelake. However, at the final root sampling date (91–96 days postplant), mean numbers of strain A1-B on these segments were approximately 0.7 log units greater at Tulelake where populations during the season were relatively stable in size (granule and seed-piece plots) or increased (drip plots) compared to South Coast, where population sizes under all three delivery regimes gradually decreased.

Populations of strain A1-B on root segments 8–16 cm distal to points of root attachment to stems were also significantly greater ($P = 0.05$) with the drip and granule delivery systems than with seed-piece inoculation. Mean population sizes (log cfu cm^{-1}) on these root segments in drip and granule plots at South Coast (21 days postplant) were 3.9 and 4.4, respectively, versus 1.8 in seed-piece inoculation plots. Values from corresponding portions of roots at Tulelake (28 days after planting) in drip, granule, and seed-piece systems were 2.8, 2.2, and 1.0 log cfu cm^{-1}, respectively. This pattern also applied to distally situated 24–32-cm root segments sampled 41 days after planting at South Coast when population sizes (log cfu cm^{-1}) for drip, granule, and seed-piece systems were 1.0, 1.0, and 0.3, respectively. Population densities of strain A1-B on distal 24–32-cm segments at Tulelake at 62 days postplant were 2.2, 1.7, and 0.3 log cfu cm^{-1} for the same three respective systems. By 91–96 days postplant, root population sizes (log cfu cm^{-1}) on distal segments (24–32 cm) in drip, granule, and seed-piece inoculation plots were 1.0, 0.3, and 0.3, respectively, at South Coast, and 2.2, 1.2, and 0.2 at Tulelake.

Preplant treatment of soil with metham sodium at South Coast resulted in increased rhizosphere population densities of drip-introduced strain A1-B. Mean population sizes of strain A1-B on the initial 0–8-cm and distal 24–32-cm segments of roots sampled at 21 and 41 days postplant, respectively, from metham-sodium-treated plots were significantly greater ($P = 0.05$) by approximately 1.2 log units than those on corresponding root segments in nonfumigated drip plots described above.

The frequency with which strain A1-B was detected on individual root segments within a given sample set varied among the three delivery systems. Strain A1-B was detected on 90–100% of all root segments assayed from drip plots at both field sites, regardless of segment position relative to point of root origin. The frequencies of detection of strain A1-B on root segments in granule plots were 100% for all segments assayed at Tulelake and 90–100% for segments of 0–8 cm and 8–16 cm and 60–90% for segments of 24–32 cm at South Coast. Detection frequencies for root segments from seed-piece inoculation plots at both sites were 70–100% for 0–8-cm and 8–16-cm segments and 30% for all 24–32-cm segments.

A monitoring of strain A1-B on the advancing tips (apical 0.5 cm) of 8-cm-long roots with points of origin adjacent to seed pieces showed that mean population sizes (log cfu per root tip) of the bacterium at South Coast were significantly greater ($P = 0.05$) on root tips in drip (3.8) and granule (3.3) plots compared to plots where seed pieces were inoculated (0.9). Mean populations on tips of 16-cm-long roots were also greater in drip (1.2) and granule (0.7) plots than in seed-piece plots (0.3), but only significantly so in drip plots. Strain A1-B was detected on all tips of 8-cm-long roots assayed from drip and granule plots (10 root tips each) at South Coast, but on only 7 of 10 tips of roots this length in seed-piece

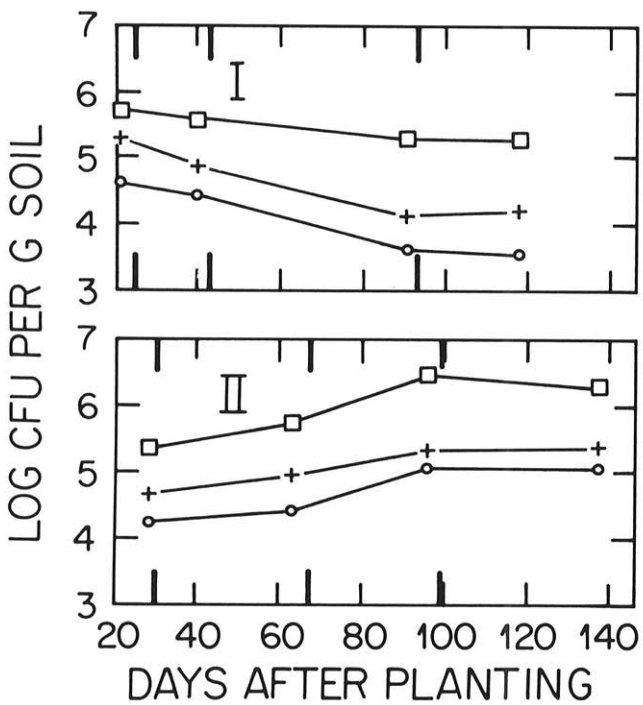

Fig. 2. Population densities of drip-irrigation-introduced *Pseudomonas fluorescens* strain A1-B in soil depth zones 15 (□) and 30 (+) cm directly beneath drip emitters and at a depth of 30 cm midway between adjacent emitters (o) at South Coast I, and Tulelake II, CA. Drip irrigation applications of strain A1-B were made at planting (0 days) at both sites and at the designated intervals (heavy line).

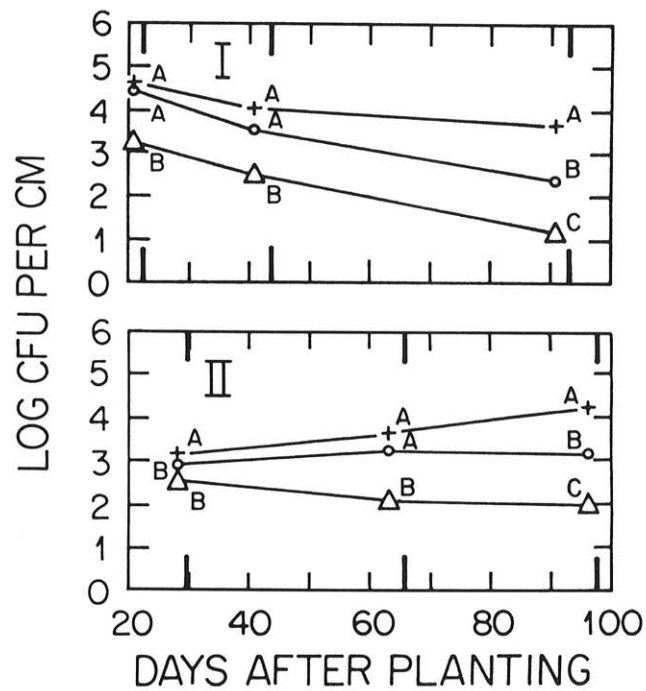

Fig. 3. Rhizosphere population densities of *Pseudomonas fluorescens* strain A1-B on the initial 0–8-cm segments of roots with points of origin adjacent to the seed pieces in plots where strain A1-B was introduced to soil by drip irrigation delivery (+), incorporation of bacteria-impregnated granules (o), and seed-piece inoculation (Δ), at South Coast I, and Tulelake II, CA. Drip applications of strain A1-B were made at planting (0 days) at both sites and at the designated intervals (heavy line).

inoculation plots. Frequencies of detection on apices of 16-cm-long roots for drip, granule, and seed-piece inoculation plots at South Coast were 6, 4, and 2 of 10 sampled tips, respectively. Root-tip population densities of strain A1-B and frequencies of detection at Tulelake were similar to those for each of the three delivery systems at South Coast.

Populations of strain A1-B on underground portions of shoots and progeny tubers. Population sizes of strain A1-B on the initial 0–8-cm segments of shoots extending above seed pieces were significantly greater ($P = 0.05$) with the drip delivery system than with seed-piece inoculation at both sites. Mean shoot populations (log cfu cm^{-1}) with drip and seed-piece delivery were 4.7 and 3.7, respectively, at South Coast, and 3.7 and 2.9 at Tulelake for the same respective systems. Average population densities (log cfu cm^{-1}) of strain A1-B on shoot segments from granule plots at Tulelake (3.6) were significantly greater than those from Tulelake seed-piece plots described above, while mean shoot populations in granule plots at South Coast (3.8) were similar to those in plots where seed pieces were inoculated (3.7).

The quantitative monitoring of populations of strain A1-B on progeny tubers arising from stolons with points of origin on shoots 0–8 cm above seed pieces (near) and from 8 cm above to just beneath the soil surface (distal) showed that the drip and granule delivery systems resulted in the establishment of large, relatively uniform populations of the bacterium on tubers regardless of tuber position in the soil. In general, tuber population densities were significantly greater ($P = 0.05$) in drip than in granule plots, and in granule than in seed-piece plots at both field sites (Fig. 4). The only exceptions to this were at the first sampling dates when populations on near tubers at South Coast in drip and granule plots were not significantly different, and at Tulelake when near tuber populations in granule plots were greater than in seed-piece plots, but not by a significant margin. Population densities of strain A1-B on tubers (near and distal) in granule and drip plots at the first sampling date were approximately 1 log unit greater in the sandy loam soil at South Coast than in corresponding plots in the silty clay loam at Tulelake. However, tuber populations in drip and granule plots at Tulelake tended to increase during the season and were approximately 1 log unit greater by harvest time than in the same plots at South Coast where population sizes remained stable (drip) or decreased slightly (granule).

Population sizes and dynamics on near tubers in seed-piece inoculation plots were similar at South Coast and Tulelake (Fig.4). In contrast, average numbers of strain A1-B on distal tubers during the season were approximately 1 log unit higher in the sandy loam soil at South Coast than in the silty clay loam at Tulelake. Mean distal tuber population sizes (log cfu g^{-1} dry weight periderm) in seed-piece inoculation plots at South Coast were 2.1, 1.0, and 1.1 at 41, 91, and 119 (harvest) days after planting, respectively, compared to values of 0.9, 0.4, and 0 after 63, 96, and 138 days, respectively, at Tulelake.

The number of tubers in a given sample set with detectable populations of strain A1-B was substantially greater in drip and granule plots than in plots where seed pieces were inoculated. Strain A1-B was detected on all tubers (near and distal) assayed from drip plots at both field sites and on all tubers sampled from Tulelake granule plots. Frequencies of detection of strain A1-B on near and distal tubers in granule plots at South Coast averaged 85 and 80%, respectively, during the season. Season-long, average frequencies of detection on near and distal tubers in seed-piece inoculation plots at South Coast were 65 and 40%, respectively, and 60 and 30% for tubers of the same respective positions in corresponding plots at Tulelake. Mean population sizes of drip-applied strain A1-B on progeny tubers in plots treated preplant with metham sodium were significantly greater ($P = 0.05$) during the season by approximately 0.7 log units compared to populations on tubers in nonfumigated drip plots described above.

Populations of strain A1-B relative to total aerobic and fluorescent pseudomonad bacteria. Delivery of strain A1-B to soil by drip irrigation injection and granule incorporation resulted in high population densities of the bacterium on roots and progeny tubers and in nonrhizosphere soil relative to total detectable aerobic bacteria and fluorescent pseudomonads (Fig. 5). Populations of strain A1-B in nonrhizosphere soil approximately 15 cm beneath the surface in drip plots at South Coast accounted for 1 and 630% of the average numbers of total detectable aerobic bacteria and native fluorescent pseudomonads, respectively, present in this portion of the soil profile during the season. Population densities of strain A1-B in nonrhizosphere soil spanning the length of the beds beneath the zone of granule incorporation (depth approximately 15 cm) at South Coast were <0.01% relative to numbers of total aerobic bacteria and accounted for 0.3% of the fluorescent pseudomonad population. Populations of strain A1-B relative to these other bacterial groups in nonrhizosphere soil, approximately 5 cm beneath inoculated seed pieces, were similar to those found from beneath zones of granule incorporation.

Rhizosphere populations of strain A1-B on the initial 0–8-cm segments of roots in drip, granule, and seed-piece inoculation plots at South Coast constituted 0.22, 0.17, and 0.01%, respectively, of the total detectable aerobic bacterial component of these root segments and 35, 27, and 1.7% of the fluorescent pseudomonads for the same respective systems of delivery (Fig. 5). Average

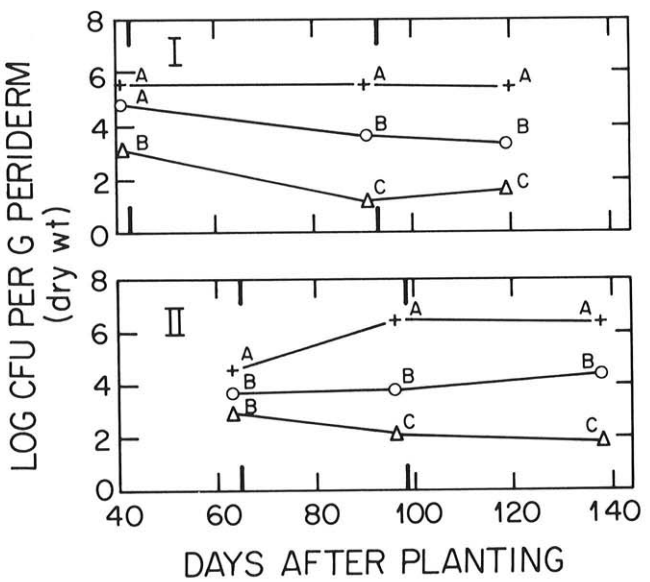

Fig. 4. Population densities of *Pseudomonas fluorescens* strain A1-B on progeny tubers arising from stolons with points of origin adjacent to the seed pieces in plots where strain A1-B was introduced to soil by drip irrigation delivery (+), incorporation of bacteria-impregnated granules (o), and seed-piece inoculation (Δ), at South Coast I, and Tulelake II, CA. Drip applications of strain A1-B were made at planting (both sites), 21 and 28 days postplant at South Coast and Tulelake, respectively, and at the designated intervals (heavy line).

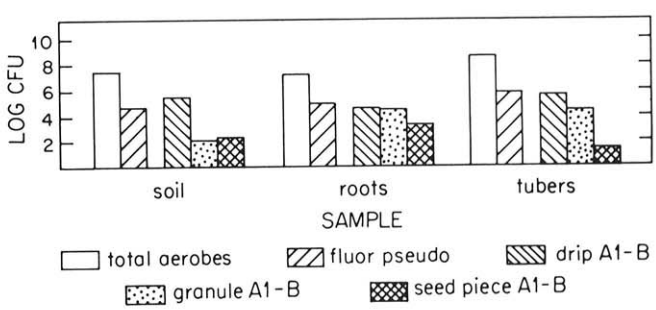

Fig. 5. Average, season-long population densities of *Pseudomonas fluorescens* strain A1-B in nonrhizosphere soil, in the rhizosphere (roots), and on progeny tubers relative to total detectable aerobic bacteria and fluorescent pseudomonads in plots where strain A1-B was introduced to soil by drip irrigation delivery, incorporation of bacteria-impregnated granules, and seed-piece inoculation at South Coast, CA.

population densities of strain A1-B on near progeny tubers during all stages of tuber development in drip and granule plots at South Coast accounted for 0.08 and 0.01%, respectively, of the total aerobic bacteria on tubers and 64 and 4% of the fluorescent pseudomonads for the same respective treatments. Strain A1-B populations on progeny tubers in seed-piece inoculation plots at South Coast represented <0.01% of these other bacterial groups. Population sizes of strain A1-B on roots and progeny tubers and in nonrhizosphere soil relative to numbers of total aerobic bacteria and fluorescent pseudomonads at Tulelake were proportionately similar to those at South Coast for each of the three delivery systems.

Distribution of strain T-1-R9 in nonrhizosphere soil and on below-ground plant parts. Conidia of *T. viride* strain T-1-R9 were distributed by drip irrigation delivery throughout the top 30 cm of soil in a pattern of decreasing density beneath emitters (Fig. 6). Average population densities of fungal propagules in samples taken at soil depth zones 15 and 30 cm directly below emitters and 30 cm midway between adjacent emitters just before the second and third drip applications of T-1-R9 and at harvest were approximately 3 log units greater in the silty clay loam soil at Tulelake than in the sandy loam at South Coast. More frequent sampling at South Coast revealed fluctuations in soil population sizes of T-1-R9 before and after drip applications. This was not detected at Tulelake where samples were not taken immediately after treatments. The types of propagules (conidia or chlamydospores) from which colonies arose were not determined. *Trichoderma* colonies were not detected on PDA-benomyl platings of soil samples from nontreated plots at either field location.

Mean rhizosphere population sizes (log cfu cm^{-1}) of drip-delivered T-1-R9 on the initial 0–8-cm segments of potato roots at Tulelake were 1.7 and 1.6 at 28 and 96 days postplant, respectively.

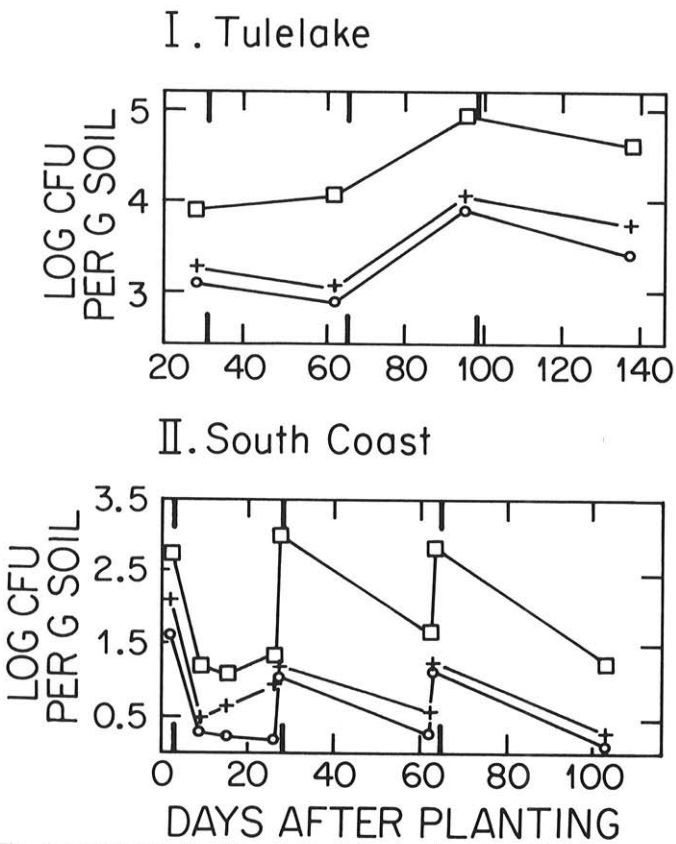

Fig. 6. Population densities of drip-irrigation-introduced *Trichoderma viride* strain T-1-R9 in soil depth zones 15 (□) and 30 (+) cm directly beneath drip emitters and at a depth of 30 cm midway between adjacent emitters (o) at Tulelake I, and South Coast II, CA. Drip irrigation applications of T-1-R9 conidia were made at planting (0 days) at both sites and at the designated intervals (heavy line).

Populations on distal 24–32-cm segments at 96 days averaged 0.9 log cfu cm^{-1}. Mean tomato root populations of T-1-R9 in drip plots at South Coast (1986) were 1.8 log cfu cm^{-1} root (0–8-cm root segments) and 2.9 log cfu cm^{-1} (8–16-cm segments) at 26 and 62 days after planting, respectively.

Population densities of drip-applied strain T-1-R9 on the initial 0–8 cm of shoots extending above the seed pieces 28 days after planting at Tulelake averaged 2.4 log cfu cm^{-1}. Populations on progeny tubers (log cfu g^{-1} dry weight of periderm) steadily increased during the season with mean values of 4.4, 4.6, and 5.0 at 63, 96, and 138 (harvest) days postplant.

DISCUSSION

These studies showed that bacterial inoculants can be effectively distributed to large volumes of soil and to underground plant parts by incorporation of bacteria-impregnated granules or injection through a low-pressure drip irrigation system. With *P. fluorescens* strain A1-B, this resulted in the establishment of persistent, high population densities on potato organs growing throughout the top approximately 35 cm of soil at two field sites of distinctly different soil type, as shown in Figure 7. The population densities and uniformity of colonization were much greater than with seed-piece inoculation. However, each technique delivered a different amount of inoculum. The colonization patterns resulting from granule incorporation and drip irrigation delivery were presumably caused by the complementary action of several mechanisms. In granule plots, elongating roots, shoots, and progeny tubers invaded soil infested with strain A1-B cells originating from granules that served as a reservoir for their continuous release. With drip delivery, the relatively uniform colonization of plant parts at high population densities presumably resulted from continual interception of high numbers of A1-B cells previously deposited in soil and by periodic bathing of plant parts with the bacterium during drip applications. Subsequent flushing action of irrigation water would also redistribute bacterial populations along plant surfaces (1).

The decline of inoculum load over time in bacteria-impregnated granules incorporated into soil appeared to be attributable to the flushing action of water and bacterial death within granules. Laboratory experiments designed to simulate field irrigations indicated that relatively high populations of strain A1-B (10^5 cfu g^{-1}) were transported into granule-free soil beneath zones of granule incorporation with each application of water. Bacterial release experiments done in nonagitated water in the laboratory and routine assay of total bacterial density of granules by vigorous washing indicate that rates of bacterial release from granules formulated in this way are moderately slow in the absence of water movement, but are moderately fast when washing action occurs. In the field, strain A1-B was detected in soil below granule-incorporated zones spanning the length of the beds in plots at both field sites. The finding of large, well-distributed populations on all underground plant parts also suggests that high numbers of bacteria were transferred from granules. The recent development of synthetic inoculant carriers such as slow-release alginate beads may provide additional techniques for the effective introduction of rhizobacteria to soil and plant parts (2).

Population densities of strain A1-B on roots in granule and drip plots in the sandy loam soil at South Coast were greater at the first sampling date than corresponding values in the silty clay loam at Tulelake. However, population sizes in the silty clay loam soil tended to gradually increase during the season, whereas populations slowly declined in the sandy loam. Nonrhizosphere soil populations of drip-introduced strain A1-B behaved similarly. This may be because the smaller soil pore size and increased adsorption of strain A1-B to soil colloids in the silty clay loam (16) impeded passive movement in downward percolating irrigation water by reducing the soil hydraulic conductivity (4) and restricting passage of bacterial cells. Previous studies have demonstrated that finely textured soils act as both filter and adsorbent for bacteria (3) and that, when the rate of soil water movement is reduced, passive distribution of bacteria is minimized (6). These factors are

probably responsible for the more relatively localized initial distribution patterns of the bacterium in soil beneath emitters at Tulelake than at South Coast during the first drip application at planting (4-hr cycle). Large initial root population densities that declined during the season in granule plots at South Coast conversely may have been caused by the effective release and distribution of high numbers of strain A1-B from granules during the first sprinkler irrigation; subsequent irrigations in the sandy loam would likely have a greater flushing effect in removing bacteria from roots and adjacent soil than in the silty clay loam

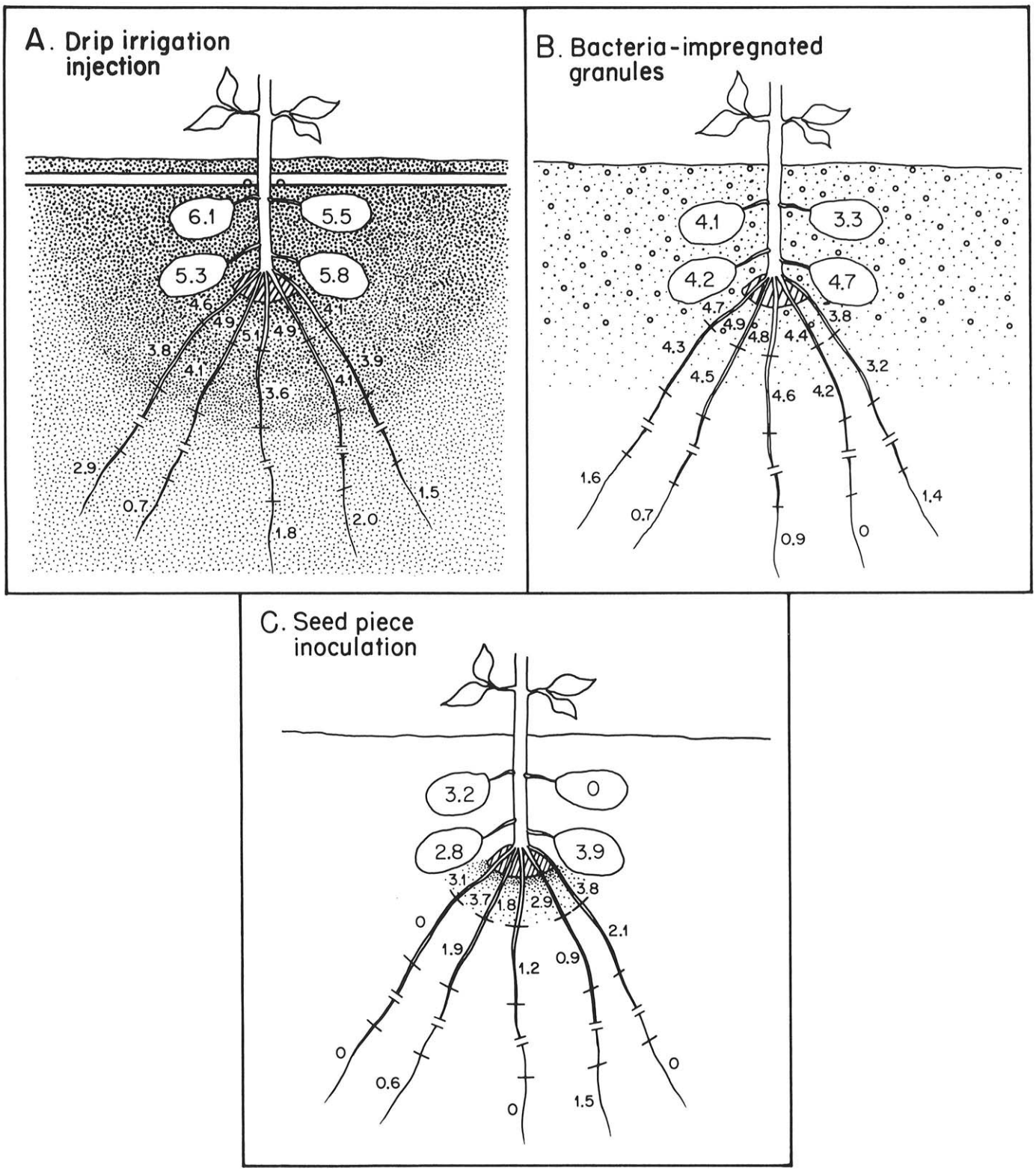

Fig. 7. Stylized depiction of the distribution patterns and population densities of *Pseudomonas fluorescens* strain A1-B on potato root segments 0–8, 8–16, and 24–32 cm distal to point of root origin (log cfu cm^{-1}) and on progeny tubers (log cfu g^{-1} dry weight periderm) following delivery of the bacterium to soil by **A,** drip irrigation, **B,** incorporation of bacteria-impregnated granules (depicted as open circles), and **C,** seed-piece inoculation. Note release of strain A1-B (depicted as dots) from granules into the surrounding soil during irrigations and drip irrigation distribution of A1-B throughout the beds at mean population densities of approximately 10^5 cfu g^{-1} soil. Also note much greater magnitude and uniformity of colonization, particularly of plant parts distal to the seed pieces, with the granule and drip systems compared to seed-piece inoculation.

soil. This also may have contributed to the substantially smaller population densities of drip-introduced *T. viride* strain T-1-R9 in nonrhizosphere soil at South Coast compared to Tulelake.

A primary objective for those interested in manipulating the rhizosphere by inoculating plant parts with root colonists has been to develop techniques and strategies to ecologically favor their growth and survival. This is intended to promote a relatively uniform, high population density on plant parts including the root tip. We view the improved spatial-temporal colonization patterns of strain A1-B on underground plant parts resulting from site-directed delivery of inoculum by the drip and granule techniques as one of presumably many tactics that can be developed to improve the efficacious use of rhizobacteria to promote root health. The specific technique selected for inoculum delivery will vary depending upon the targeted sphere of influence, the crop, cropping system, and desired benefit. For example, the water injection and granule techniques have major advantages over the present seed inoculation methods in promoting bacterial colonization of root tips. This is of importance when considering the control of fungal pathogens such as *Fusarium* and *Verticillium* spp. or plant parasitic nematodes like *Meloidogyne* spp. that invade the advancing root tip throughout the season.

The low-pressure drip injection system for delivering inoculum provides unique opportunities to use rhizobacteria to control specific diseases. As shown with *T. viride*, it also can be used to effectively deliver spores of fungal antagonists to the soil and rhizosphere. The system offers great flexibility in that liquid inoculum can be used, it can be applied repeatedly at any time during plant growth, and selective substrates to specifically encourage the growth of the antagonist can also be added with the inoculum (J. Bahme, *unpublished*). It also is possible to combine the use of antagonists with pesticides. For example, benomyl-resistant *Trichoderma* could be added with benomyl. Data on the elevated population sizes of strain A1-B on potato plant parts following preplant soil treatment with metham sodium further indicate the potential for combining chemical and biological treatments to effect changes in soil microflora.

LITERATURE CITED

1. Bahme, J. B., and Schroth, M. N. 1987. Spatial-temporal colonization patterns of a rhizobacterium on underground organs of potato. Phytopathology 77:1093-1100.
2. Bashan, Y. 1986. Alginate beads as synthetic inoculant carriers for slow release of bacteria that affect plant growth. Appl. Environ. Microbiol. 51:1089-1098.
3. Bitton, G., Lahav, N., and Henis, Y. 1974. Movement and retention of *Klebsiella aerogenes* in soil columns. Plant Soil 40:373-380.
4. Brady, N. C. 1984. Soil water: Characteristics and behavior. Pages 73-108 in: The Nature and Properties of Soils. Macmillan Publishing Co., New York. 750 pp.
5. Foster, R. C., Rovira, A. D., and Cock, T. W. 1983. Ultrastructure of the Root-Soil Interface. The American Phytopathological Society, St. Paul, MN. 157 pp.
6. Frazier, W. C., and Fred, E. B. 1921. Movement of legume bacteria in soil. Soil Sci. 14:29-31.
7. Gould, W. D., Hagedorn, C, Bardinelli, T. R., and Zablotowicz, R. M. 1985. New selective media for enumeration and recovery of fluorescent pseudomonads from various habitats. Appl. Environ. Microbiol. 49:No.1.
8. Howell, C. R., and Stipanovic, R. D. 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69:480-482.
9. Howell, C. R., and Stipanovic, R. D. 1980. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. Phytopathology 70:712-715.
10. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
11. Kloepper, J. W., and Schroth, M. N. 1981. Relationship of in vitro antibiosis of plant growth-promoting rhizobacteria to plant growth and displacement of root microflora. Phytopathology 71:1020-1024.
12. Kloepper, J. W., and Schroth, M. N. 1981. Development of a powder formulation of rhizobacteria for inoculation of potato seed pieces. Phytopathology 71:590-592.
13. Kloepper, J. W., Schroth, M. N., and Miller, T. D. 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70:1078-1082.
14. Loper, J. E., Haack, C., and Schroth, M. N. 1984. Population dynamics of soil pseudomonads in the rhizosphere of potato (*Solanum tuberosum* L.). Appl. Environ. Microbiol. 49:416-422.
15. Loper, J. E., Suslow, T. V., and Schroth, M. N. 1984. Lognormal distribution of bacterial populations in the rhizosphere. Phytopathology 74:1454-1460.
16. Marshall, K. C. 1971. Sorptive interactions between soil particles and microorganisms. Pages 409-445 in: Soil Biochemistry. Vol. 2. A. D. McLaren and J. J. Skujins, eds. Marcell Dekker, Inc. N.Y.
17. Papavizas, G. C., and Lewis, J. A. 1983. Physiological and biocontrol characteristics of stable mutants of *Trichoderma viride* resistant to MBC fungicides. Phytopathology 73:407-411.
18. Suslow, T. V., and Schroth, M. N. 1982. Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. Phytopathology 72:111-115.
19. Van Gundy, S. D., and Garabedian, S. 1984. Application of nematicides through drip irrigation systems. Med. Fac. Landbouww. Rijksuniv. Gent. 49/2b:629-634.
20. Weller, D. M. 1984. Distribution of a take-all suppressive strain of *Pseudomonas fluorescens* on seminal roots of winter wheat. Appl. Environ. Microbiol. 48:897-899.
21. Weller, D. M., and Cook, R. J. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73:463-469.
22. Yuen, G. Y., and Schroth, M. N. 1986. Inhibition of *Fusarium oxysporum* f. sp. *dianthi* by iron competition with an *Alcaligenes* sp. Phytopathology 76:171-176.
23. Yuen, G. Y., and Schroth, M. N. 1986. Interactions of *Pseudomonas fluorescens* strain E6 with ornamental plants and its effect on the composition of root-colonizing microflora. Phytopathology 76:176-180.