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Aerial Dispersal and Epiphytic Survival of *Pseudomonas syringae* during a Pretest for the Release of Genetically Engineered Strains into the Environment

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Prospective experimental field evaluation of genetically engineered microorganisms, such as microbial pest control agents, raises issues of how to properly ascertain their fate and survival in the environment. Field trials with recombinant organisms must reflect requirements for sampling and monitoring. Field trials were conducted at Tulelake, Calif., to monitor the numbers of viable cells of a nonrecombinant strain of *Pseudomonas syringae* that entered the atmosphere and landed on plants and soil during and after an aerosol spray application. An exponential decrease in numbers of viable cells deposited at increasing distances from three sprayed plots was observed. The relative rate of survival of cells sprayed directly on plants was more than 10 times higher than that of cells dispersed through the air to similar adjacent plants. Results are being used to gain experience with the characteristics of a release site that influence containment or dispersal and to develop appropriate sampling methodologies for evaluating survival and dispersal characteristics of genetically engineered bacteria released into the environment. The ability to make predictions about microbial dispersal and survival will reduce the uncertainties associated with environmental releases of recombinant organisms.

Bacteria, fungi, and viruses have been extensively tested and, in several instances, used commercially to control a variety of agricultural pests, including insects, plant pathogens, and weeds (1, 3, 9, 13, 14, 16, 18, 20, 22). Recombinant DNA technology offers the possibility of genetically modifying microbial pest control agents to improve their performance (11, 15). Expression of novel phenotypes in bacteria as a result of recombinant DNA techniques may lead to development of enhanced competitive ability (e.g., ability to utilize new substrates), enhanced environmental fitness, enhanced efficacy against target organisms, and other valuable characteristics. Alternatively, genes that determine deleterious traits can be specifically deleted to produce environmentally competent microorganisms which might effectively exclude their deleterious counterparts in natural settings (15).

Applicants have requested experimental-use permits from the U.S. Environmental Protection Agency to release genetically engineered microbes in small-scale field trials. Concerns about possible environmental effects of genetically engineered microorganisms include uncertainty about their dispersal, survival, and possible interactions with indigenous organisms. Researchers, as well as regulatory agencies, must have the tools to assess possible risks associated with the use of these microbial agents.

In the case of bacteria applied to crops in aerosol form, important issues include how to ascertain their dispersal through the atmosphere and survival in unintended habitats after deposition. Specific research needs include having the ability to predict numbers of viable microorganisms that enter the atmosphere during the test and estimating their downwind aerial concentrations; determining the growth or survival (or both) characteristics of inoculated and dispersed organisms; establishing appropriate numbers of organisms and methods of application and defining characteristics of the site that would influence containment or dispersal; developing appropriate sampling methodologies for the release, including a determination of factors that influence detection, limits of detection, periodicity of sampling, and the rationale for monitoring procedures; and designing appropriate contingency plans to terminate the experiment, if necessary, and to eradicate the applied organism. Experiments to address all but the last two needs are described here and should add to a useful data base for decision making by regulatory agencies. The ability to make predictions about microbial dispersal and survival will reduce the uncertainty associated with environmental release of recombinant organisms.

Most planned releases are for applications of microbial pest control agents to agricultural crops. Research with strains of *Pseudomonas syringae* and other potential pest control agents has progressed to the point at which field tests are necessary to effectively evaluate their performance and to address basic ecological questions, such as the specificity of competition among bacteria. In this paper, we report the dispersal, survival, and short-term population dynamics of a nonrecombinant strain of *P. syringae* at a site where releases of an Ice$^-$ strain of this species constructed in vitro (14) are

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scheduled. We discuss how these results will be used in the design and refinement of environmental release trials, with an emphasis on detection and monitoring of recombinant bacteria.

MATERIALS AND METHODS

Organisms and culture media. *P. syringae* MF714R was used in all experiments. This organism is a nonrecombinant, ice nucleation-active strain originally isolated from healthy almond leaves near Fresno, Calif. A spontaneous mutant resistant to the antibiotic rifampin was selected in culture and verified to have growth and survival characteristics similar to those of the parental rifampin-sensitive strain. This strain was tested for pathogenicity and shown to be avirulent on all plants tested, including bean (*Phaseolus vulgaris* L.) cv. Eagle; tomato (*Lycopersicon esculentum* L.) cv. Bonny Best; oat (*Avena sativa* L.) cv. White Rose; cucumber (*Cucumis salivar* L.) cv. National Pickling; pea (*Pisum sativum* L.) cv. Spring; corn (*Zea mays* L.) cv. PX20; pear (*Pyrus communis* L.) cv. Bartlett; and navel orange (*Citrus sinensis* L.) cv. Washington. Leaves and stems of all plants and 1-cm-diameter pear fruits were infiltrated with a cell suspension of ca. 10^6 *P. syringae* cells per ml with a needle. An inoculum of strain MF714R for pathogenicity studies and field inoculations was grown for 2 days at 24°C on King medium B (8) containing 100 μg of rifampin per ml and 100 μg of cycloheximide per ml. Cells were then scraped with a sterile spatula from the surfaces of plates and suspended in and diluted to appropriate concentrations with sterile distilled water.

A medium highly selective for strain MF714R (SSMR) was used to recover this strain from field samples. SSMR contained (per liter of distilled water) 12.0 g of sorbitol, 0.8 g of $K_2HPO_4 \cdot 3H_2O$, 0.8 g of $KH_2PO_4 \cdot 3H_2O$, 0.13 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of L-histidine, 128 mg of cetrimide, 100 mg of rifampin, 100 mg of cycloheximide, 50 mg of benomyl (Benlate; E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.), and 15.0 g of Bacto-Agar (pH 7.0; Difco Laboratories, Detroit, Mich.). Strain MF714R had a plating efficiency of 70 to 90% on this medium compared with King medium B.

Field test site. The field test site was at the University of California Agricultural Field Station, near Tulelake, Calif. The research station is located at the approximate center of the Tulelake Basin in northern California, which is high desert, irrigated, and heavily agricultural. The overall dimensions of the test site were approximately 120 by 70 m. Three experimental plots of oats, cv. Cayuse, were established, each measuring about 7 by 7 m and separated by a bare-soil buffer zone of approximately 23 m (Fig. 1). Seeds were planted ca. 2 cm apart in rows separated by ca. 10 cm. Plants were ca. 5 to 10 cm high at the time of inoculation. The plots (7 by 7 m) were arranged in a line such that prevailing winds blew perpendicularly to the main axis, to minimize contamination of one plot by another. A biological net of oats was also established downwind of the plots for sampling purposes (Fig. 1). The biological net consisted of rows of oats (about 1.5 m long), the centers of which were planted at distances of 3, 6, and 9 m away from plots A and C, as well as at 15, 21, 27, and 33 m away from plot B along the perpendicular axis. The oats in these downwind locations were planted at the same time and density as the oats in the experimental plots and were separated by bare soil.

Meteorological equipment. A tower containing meteorological equipment was centrally located on the southern border of plot B. Air temperature, relative humidity, solar radiation, and wind speed and direction were all measured at 1-min intervals on sampling event days. Wind speed and direction and air temperature were measured at the plant canopy and 0.5, 1, 3, and 10 m above the ground. At the 3-m level, wind speed and direction were measured with a bivane, whereas at the other heights standard wind speed and direction equipment were used. Humidity and solar radiation data were collected at the 2-m level.

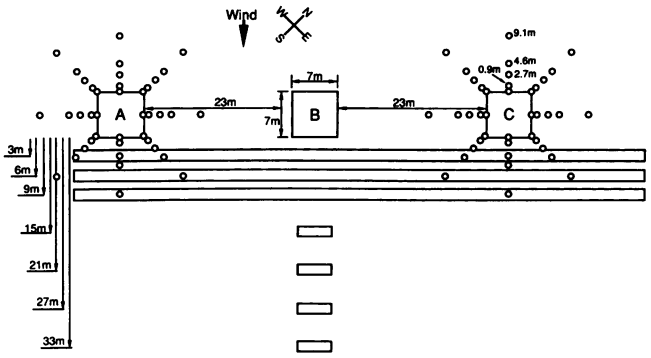

FIG. 1. Design of the field plots, illustrating the locations of plots 1, 2, and 3 (A, B, and C, respectively), the biological net of oats (rectangular areas), and sampling stations with petri dishes (○). For clarity, the sampling stations around plot 2 are not shown, but they were identical to those of plots 1 and 3.

Application of bacteria to plots. Bacteria were sprayed onto plants in the plots with a CO_2-pressurized, hand-held sprayer equipped with a large-orifice (Tee-Jet 8004) nozzle operated at a pressure of 2,800 g/cm^2 to produce large spray droplets. The spray nozzle was kept close to plants (15 to 20 cm) to minimize aerosol movement of bacteria from the site of application. Plot 1 received approximately the concentration of bacteria (8.1×10^7 CFU/ml) planned for releases of recombinant organisms. Plot 2 received 6.4×10^8 CFU/ml, and plot 3 received 1.8×10^{10} CFU/ml. These three concentrations each constituted a treatment and were designed to ascertain a threshold for bacterial detection at different distances from the source and times after spraying. Concentrations of bacteria sprayed were determined by plate counts of serially diluted bacterial suspensions. Spraying of each plot was done under relatively calm wind conditions, and plants were sprayed to runoff (approximately 4 liters per plot). Plot 1 was sprayed on 14 July 1986 between 8:43 and 8:46 p.m., plot 2 was sprayed on the same day between 8:54 and 8:57 p.m., and plot 3 was sprayed on 16 July between 5:36 and 5:40 a.m. A single application of strain MF714R was made in each plot at the designated cell concentration.

Measurement of deposition of bacteria. Ground-level deposition of bacteria from the airstream was monitored with 10-cm-diameter petri dishes containing SSMR agar that were placed on the ground in a spokelike formation emanating at equal angles from the edge of each plot at distances of 0, 0.9, 2.7, 4.6, and 9.1 m (Fig. 1). A series of plates was opened in each plot as controls for 30 min at about 6 h before spraying. Plates were opened just before spray inoculation of each plot and closed 20 min after cessation of inoculation (total exposure to atmosphere, ca. 30 min). Additional plates were also exposed for 30 min at each of the sampling positions in plots 1 and 2 at 17 h after spraying (during gusty midday breezes) and again at 32 h after spraying (during calm winds). Colonies were counted after 5 days of incubation at 28°C.

Enumerating bacteria from plant surfaces and soil. Each

TABLE 1. Meteorological conditions during spray application of nonrecombinant *P. syringae* to plots 1, 2, and 3

Plot no.	Time when data were recorded (h)	Mean wind speed m/s at[a]:				Maximum/minimum speed (m/s) at 1 m	Relative humidity (%)	Temp (°C)
		1 m	2 m	3 m	10 m			
1	20:43–21:13	1.5	1.8	1.9	3.1	2/1	57	8.5
2	20:54–21:24	1.3	1.5	1.8	2.9	2/0	60	8.5
3	05:34–06:04	0.7	0.8	1.0	1.3	1/0	101	5.7

[a] The wind was westerly at all three plots.

replicate sample of leaves consisted of 30 individual oat plants (each ca. 0.5 g) excised about 1 cm above soil level. Plants were selected randomly from within each sprayed plot or from sampling sites located downwind from inoculated plants. Four replicate samples of leaves were collected into sterile plastic bags from each sampling location at 6 h before spraying, 1 h after spraying, and at intervals of 1 to 7 days for up to 16 days after spraying. Samples of soil, each totaling 30 g, were collected at a depth of 0 to 3 cm from randomly chosen locations within each sprayed plot each time leaves were sampled. Leaves were weighed, submerged in 100 ml of sterile washing buffer consisting of 0.1 M potassium phosphate buffer containing 0.1% Bacto-Peptone (Difco), pH 7.0, and sonicated for 7 min in a Bransonic 52 ultrasonic cleaner (Branson Sonic Power Co., Danbury, Conn.) at 20°C to remove cells from leaves. This method recovers at least 70% of the total bacteria, determined by dilution plating of macerated tissue from samples exposed to different environments (R. D. O'Brien and S. E. Lindow, manuscript submitted). Tenfold serial dilutions of leaf washings were plated on SSMR and King medium B. Undiluted leaf washings were plated on three replicate SSMR plates for all samples except those from within the inoculated plots to increase the sensitivity of detection of strain MF714R. Soil samples were processed in a manner similar to that used for leaf samples, except that each 30-g soil sample was reduced to small particles by rubbing within the plastic sampling bags before sonication. Samples collected before inoculation and at 1 and 17 h after inoculation of plots 1 and 2 were processed within 1 h after collection at a laboratory at the Tulelake field station. All other samples were collected, cooled to 4°C, placed in insulated containers with cooling blocks, shipped to Berkeley, Calif., refrigerated at 4°C, and processed within 18 h of sampling. Colonies on SSMR were enumerated after 5 days of incubation at 28°C. A subset of colonies recovered on SSMR was verified as being strain MF714R by testing for ice nucleation activity by a replica freezing assay described previously (17). Only colonies that resembled strain MF714R on SSMR and had an Ice^+ phenotype were recorded as positive counts for MF714R. Statistical computations were done with software provided by Statistical Analysis Systems (SAS Institute Inc., Cary, N.C.). The general linear model procedure was used to calculate the means and standard errors of log-transformed population sizes.

RESULTS

Deposition of bacteria via aerosols. Overall, variable (0.73 to 1.5 m/s) westerly winds and cool temperatures (5 to 9°C) prevailed during spraying of the test plots (Table 1). Because of the gentle westerly flow of air during spraying, an asymmetric pattern of bacterial dispersal from the spray sites was observed in the general downwind direction (Fig. 2). Air movement was lowest during the early morning period in which plot 3 was sprayed. The highest bacterial counts (>2,000 CFU per plate) were obtained within plot boundaries or close to and downwind from plots. Few bacteria were detected more than 1 m upwind of any inoculated plot. Cells of strain MF714R were detected at each of the farthest downwind gravity stations (9.1 m) within 20 min of the spray application. Whereas relatively high numbers of strain MF714R were detected close to the site of inoculation (from 10 to 66 CFU/cm^2), the number of cells deposited per unit area decreased greatly with increasing distance from inoculated plants. The number of colonies deposited per petri dish (Fig. 2) was transformed to number of cells deposited per square centimeter (N), and best-fit relationships between N and distance (D; in meters) from the edge of the plot were determined by least-squares regression analyses. N was best related to D by the exponential decay function $N = Ae^{-XD}$. When the deposition density, N, was averaged over all directions for each distance from the site of inoculation, X assumed the values 0.45, 0.36, and 0.30 and A assumed the values 24.8, 17.4, and 29.2 ($R = -0.99$, -0.99, and -0.98) for plots 1, 2, and 3, respectively. When only the average deposition density in the southern (downwind) quadrant was averaged for each distance from the site of inoculation, X assumed the values 0.59, 0.90, and 0.54 and A assumed the values 135.0, 164.5, and 141.2 ($R = -0.97$, -0.99, -0.98) for plots 1, 2, and 3, respectively. Other possible relationships between N and D tested, including $N = A + XD$, $N = AD^X$, and $N = A + \ln D$, did not describe the distribution of viable cells as well as did the relationship described above. No viable cells of strain MF714R were detected on deposition plates placed around sprayed plots at any time before or after the initial foliar spray events.

Survival of bacteria in and around sprayed plots. Oat plants sprayed with a suspension of *P. syringae* MF714R supported large and relatively constant population sizes of this strain for up to 16 days after inoculation in all three plots (Fig. 3 to 5). Although plots 2 and 3 were inoculated with cell suspensions about 10- and 100-fold more concentrated than those used for plot 1, the number of viable cells recovered from plants immediately after inoculation varied by less than

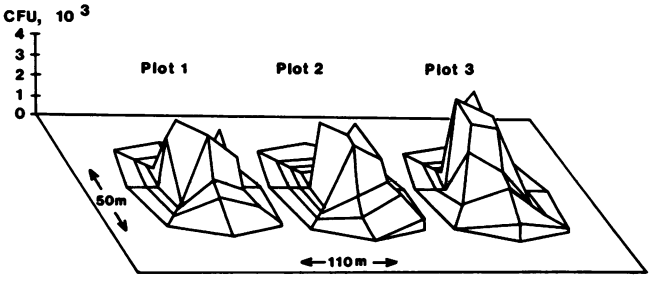

FIG. 2. Distribution of CFU of *P. syringae* MF714R which landed in petri dishes located at the positions indicated in Fig. 1. Plots were sprayed with 8.1×10^7 (plot 1), 6.4×10^8 (plot 2), and 1.8×10^{10} (plot 3) cells per ml.

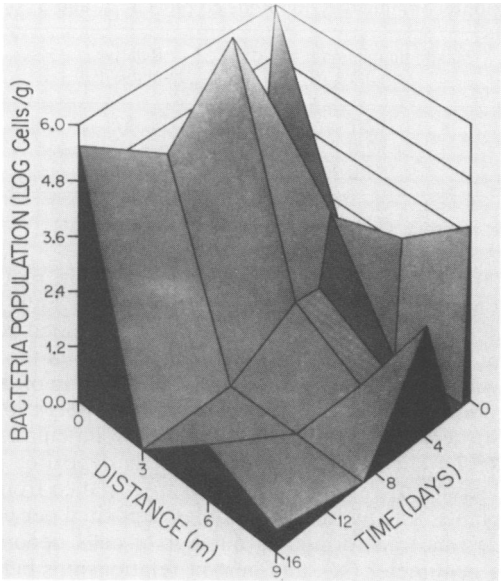

FIG. 3. Sizes of *P. syringae* MF714R populations on oat plants within plot 1 and at different distances from the edge of the plot after spray application of 8.1×10^7 cells per ml. Illustrated are populations immediately after spraying (0 days) and at times up to 16 days after spraying.

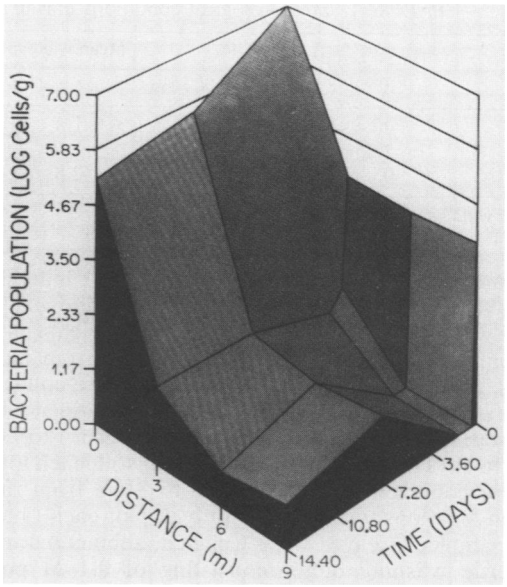

FIG. 5. Sizes of *P. syringae* MF714R populations on oat plants within plot 3 and at different distances from the edge of the plot after spray application of 1.8×10^{10} cells per ml. Samples were taken immediately after spraying (0 days) and at times up to 14.4 days after spraying.

5-fold. Population sizes decreased by 4- to 10-fold within 7 days after spraying. Counts varied significantly between sampling dates within a plot but generally did not differ between plots on a given sampling date (Fig. 3 to 5). Strain MF714R generally maintained a population size on inoculated plants in excess of 10^5 cells per g of fresh weight in all plots and on all sampling dates. No rifampin-resistant bacteria were detected on oat plants plated on SSMR before inoculation with strain MF714R (data not shown).

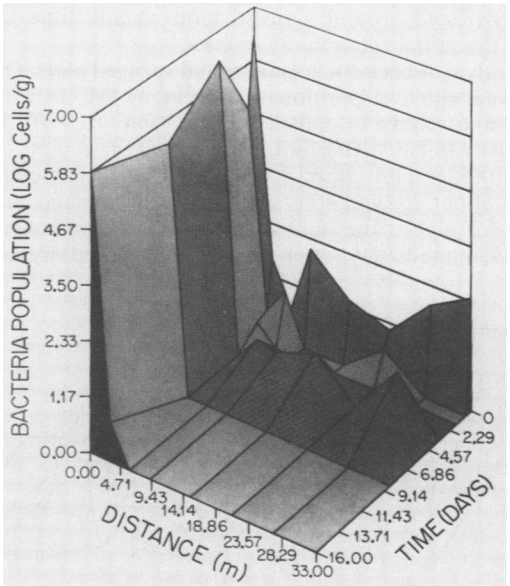

FIG. 4. Sizes of *P. syringae* MF714R populations on oat plants within plot 2 and at different distances from the edge of the plot after spray application of 6.4×10^8 cells per ml. Samples were taken immediately after spraying (0 days) and at times up to 16 days after spraying.

Cells of strain MF714R were detected on oat plants up to 27 m downwind of inoculated plots immediately after spraying. The population size of MF714R on plants as close as 3 m from sprayed plots was from 100- to 1,000-fold lower than on nearby directly sprayed plants (Fig. 3 to 5). Numbers of strain MF714R on plants downwind from sprayed plots immediately after spraying generally decreased with increasing distance (from 10^2 to 10^3 cells per g of fresh weight). Numbers on plants more than 9 m from inoculated plants were always less than about 100 cells per g of fresh weight, which was close to the limit of detection. Although MF714R was detected on plants adjacent to inoculated plants immediately after spraying, numbers dropped dramatically within 1 day on nearly all plants surrounding these sprayed plots to levels at or below the detection level (ca. 10 cells per g). The population size of this strain remained less than 100 cells per g at all times and at all distances from the sprayed plots and was most frequently undetected (Fig. 3 to 5).

Large numbers of strain MF714R organisms were detected in the upper layers of soil in sprayed plots immediately after inoculation of the plants (Fig. 6). The number of cells of strain MF714R detected in soil in treated plots was approximately proportional to the concentration of cells applied to oat plants in these plots, ranging from about 300 cells per g in plot 1 to about 10^5 cells per g in plot 3 (Fig. 6). The number of viable cells of MF714R in the upper soil layers dropped rapidly to undetectable levels within about 2 days in all trials (Fig. 6).

DISCUSSION

Whereas considerable literature exists on the bacterial, fungal, and viral contents of air, much of this has been aimed

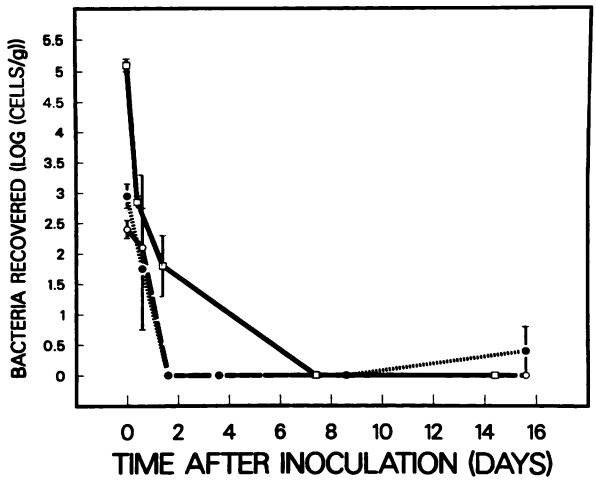

FIG. 6. Sizes of *P. syringae* MF714R populations in the top 3 cm of soil within plots of oat plants sprayed with a cell suspension of 8.1×10^7 (○), 6.4×10^8 (●), or 1.8×10^{10} (□) cells per ml immediately after inoculation and at subsequent sampling times.

at determining the spread of plant, animal, and human pathogens. Thus, most studies have been within closed buildings (4) or in the vicinity of sewage treatment plants (21) and usually have had no firm estimate of the source strength of the microbes studied or the physical properties of the aerosol. Most atmospheric studies of microbes have emphasized fungi and pollen (7), which probably are much more stable in air than are bacteria. Plants have recently been shown to be sources of aerosolized bacteria (5, 12, 19, 23), but these studies have assessed plant canopies of a large area as sources of airborne bacteria. Perombelon et al. have studied the dispersal of a point source of aerosolized *Erwinia carotovora* (19). Whereas general atmospheric features were related to dispersal of this bacterium, no good estimate of the nature of the dispersal gradient or the success of dispersed cells at colonizing new sites on plants was made. Thus, there is a surprising lack of information useful for predicting relatively short-distance dispersal patterns or the fitness of bacteria on plants after open-air inoculations.

Whereas significant numbers of *P. syringae* MF714R were detected within 10 m of plots sprayed during gentle breezes, no detectable movement of cells from the plot via aerosols occurred after inoculation. The relative concentrations of cells in the air at different distances can be assumed to be accurately estimated by the rates of deposition from the air at those distances. The number of viable cells deposited onto new habitats which can be colonized, not the absolute number of cells dispersed from the plots, is of primary importance in estimates of potential effects in such sites. The concentrations of cells deposited per unit area away from the initial areas of spray application revealed a gradient which was proportional to the inverse power of the distance from the point of application. Dispersal of fungal spores and the resultant incidence of disease also frequently exhibit a logarithmic dispersal pattern (6).

The viable cell counts in petri dishes at different distances from plots 1, 2, and 3 did not differ in proportion to the concentration of cells used to spray each plot. For example, the number and distribution pattern of viable cells from plots 1 and 2 were similar, even though the cell concentrations applied differed about eightfold (Fig. 2). These results are largely influenced by the direction and speed of air movement during spraying. A much lower mean wind speed prevailed when plot 3 was sprayed, minimizing the drift compared with those of plots 1 and 2. Differences in downwind deposition would likely have been more pronounced if the plates had also been set at distances farther from the plot. By using the exponential decay function and setting N = one cell per square centimeter, one can calculate the distance (D) at which only one cell is deposited per square centimeter. The values were 7, 8, and 11 m, respectively for plots 1, 2, and 3. If all three plots had been sprayed at the same time (and thus with identical wind conditions), the differences in D would have been more pronounced.

The median diameter of droplets produced by the nozzle used in these spray events was about 100 μm (Wes Yates, personal communication). Such a droplet has a volume of 5.2×10^{-7} ml. Thus, even at the lowest cell density sprayed onto plot 1, such a droplet would, on the average, contain at least one cell, whereas in plot 2 it would contain over 100 cells. Thus, most, if not all, droplets created during inoculation contained at least one cell and most contained several cells of MF714R. Deposition of such droplets, or dry particles created by evaporation, onto culture medium would, in many cases, result in colonies that arise from several viable cells. Therefore, the number of CFU may be lower than the number of deposited bacterial cells if all cells remained viable during dispersal. Additional evidence for this hypothesis was obtained by comparing (i) the number of viable cells of MF714R recovered from plant tissue downwind of the plot with (ii) the number of CFU deposited at such distances onto petri plates. A direct comparison at a distance of 3 to 9 m is possible on the basis of the data. Approximately 100 to 1,000 cells per g of leaf tissue (ca. 5 to 50 cells per cm^2) were deposited on plants within this distance of plot 1. However, the average concentration of MF714R deposited on petri dishes within this distance ranged from 0.4 to 22 CFU/cm^2. Similarly, whereas the deposition density of CFU on petri dishes over this distance downwind from plot 3 was similar to that of plot 1, the number of viable cells of MF714R per gram recovered from plants ranged from 10^3 to nearly 10^4/g (10^2 to 10^3 CFU/cm^2). Thus, the number of viable cells per square centimeter of plant leaf exceeded the number expected on the basis of CFU per square centimeter on petri dishes, and this difference was generally proportional to the concentration of cells used as an inoculum in spray mixtures. Trapping of cells on adjacent plants may be a more accurate method by which dispersal of cells from inoculations with very high concentrations of bacterial sprays can be assessed. However, it is unlikely that cell concentrations as high as those used in plot 3 (10^{10} cells per ml) will ever be used or required in deliberate sprays under field conditions.

Integration of the average cell deposition densities of MF714R within various distances of plot 1 revealed that, of a total of 3.4×10^{11} CFU applied to this plot, only 7.7×10^6, 4.2×10^6, and 6.0×10^6 CFU were deposited within concentric rings of 1 to 3, 3 to 5, and 5 to 10 m from this plot. The total deposition of this strain within a range of 10 m from plot 1 was 1.8×10^7, which represents only 0.005% of the total cell number applied.

Conditions were favorable for survival of strain MF714R in the 16 days after direct spray inoculation. Thus, the population sizes of MF714R on the inoculated plants in plots 1 and 2 remained more or less constant at 10^5 to 10^6 cells per g between 2 and 16 days after inoculation. The population size on plants in plot 3 declined to about 10^5 cells per g, probably since the initial high inoculum could not be supported at 10^7 cells per g. During these 2 weeks, the mass of

plants also increased about fivefold. Thus, net growth of MF714R occurred with the oats on plots 1 and 2. Whereas some bacterial strains applied to plants in the field have survived as well as MF714R (13, 16, 18), others have not (9). Knudsen and Spurr reported that populations of *P. cepacia* declined by 2 orders of magnitude over a similar 2-week period after foliar applications to peanuts (9). There are too many differences between these studies to permit a conclusive statement about why the behavior of the two *Pseudomonas* species was distinctly different. However, *P. syringae* is an epiphyte, whereas *P. cepacia* is typically found in soil-rhizosphere environments. Leaf wetness, which has a positive influence on survival of epiphytes (9), may also have contributed to differences between the levels of bacterial survival in these studies.

A significant difference between the relative survival rates of strain MF714R was noted between plants directly spray inoculated and plants to which this bacterium was dispersed by aerosolization and downwind movement of the aerosol. Population sizes of MF714R on directly sprayed plants changed very little over 16 days, remaining at 10^5 to 10^6 cells per g after inoculation, whereas population sizes decreased about 100- to 1,000-fold and more after dispersal to oat plants downwind. Thus, the number of MF714R cells on directly inoculated plants never dropped below 10 to 15% of the maximum number detected shortly after spray inoculation. However, less than 1.0 to 0.01% of cells that traveled to plants adjacent to sprayed plots survived even for 1 day. The lower rate of survival on adjacent plants may have reflected a higher death rate of cells subjected to the shock of aerosolization and downwind movement accompanied by desiccation. Small aerosol droplets of 100-μm diameter or less would evaporate rapidly under the low-humidity conditions encountered in the tests (W. Yates, personal communication). Whereas all cells of MF714R obviously did not die during such desiccation (Fig. 2), the number recovered from plants rapidly decreased with time after spraying (Fig. 3 to 5). Alternatively, the numbers of bacteria that occur naturally on oat leaves (10^5 cells per g of fresh weight) might compete more effectively with the low cell densities deposited on leaves by downwind aerosols than on leaves for which all available niches were filled by application of large numbers of MF714R cells by direct spraying. The ramifications and mechanisms of this phenomenon (dose required for successful colonization or reproduction) are significant to microbial ecology and the application of biotechnology to agriculture and should be investigated further.

The soil surface was not a major source for redistribution of strain MF714R. This organism died quickly when applied to moist soil. The rate of death in soil was similar to that reported for another *P. syringae* strain studied in soil under laboratory conditions (14). Thus, the fluctuating physical and biological environment of this field study did not cause a significant change in the predicted survival of this bacterial species.

An evaluation under field conditions of the dispersal and survival characteristics of biocontrol agents is necessary to help make biological controls a more predictable science. With the added interest in genetic manipulation of biocontrol agents, opportunities exist for significant scientific advancements in predictive microbial ecology. However, we should not forget that the fundamental parameters needed for developing a predictive ecology may not be present in the published literature.

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