

Inter-root Movement of *Azospirillum brasilense* and Subsequent Root Colonization of Crop and Weed Seedlings Growing in Soil

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Abstract. Inter-root movement and dispersion of the beneficial bacterium *Azospirillum brasilense* were monitored in root systems of wheat seedlings growing in the field and in growth chamber soil trays. Two strains were used, a motile wild-type strain (Cd, mot⁺) and a motility deficient strain (mot⁻), which was derived from the Cd strain. Root colonization by two wild-type strains (Cd and Sp-245) was studied in 64 plant species growing in pots in the greenhouse. The two wild-type strains of *A. brasilense* were capable of colonizing all tested plant species. In soil trays and in the field, mot⁺ cells moved from inoculated roots to non-inoculated roots of either wheat plants or weeds growing in the same field plot, but the mot⁻ strain did not move toward non-inoculated roots of either plant species. In the field, both mot⁺ and mot⁻ strains of *A. brasilense* survived well in the rhizosphere of wheat for 30 days, but only mot⁺ moved between different weeds, regardless of the species, botanical family, or whether they were annuals or perennials. In plant-free, water-saturated soils, either in columns or in the field, both strains remained at the inoculation site and did not move.

It is proposed (a) that *A. brasilense* is not a plant-specific bacterium and that (b) colonization of the entire root system in soil is an active process determined by bacterial motility; it is not plant specific, but depends on the presence of plants.

Introduction

Azospirillum has been used as a plant inoculant to improve plant growth and productivity [13, 24]. *Azospirillum* cells can be found in the root system of several plant species [17, 21, 25, 26, 28, 32, 36, 37, 41, 42]. When *Azospirillum* was tested under controlled conditions, bacterial movement in soil probably depended on the presence of plants [10]. In plant-free soil columns, bacteria were rapidly and

strongly absorbed into the clay and organic fractions of the soil, thereby restricting movement [11]. Root tips were shown to be efficient vectors for passive vertical transfer of *A. brasilense* [12]. In the planted field, cells originating from soil surface inoculation could be found as deep as 50 cm and as far as 30 cm from the original inoculation site [10, 14]. Thus, bacterial motility should be considered an important element in *Azospirillum* technology [22, 43].

In vitro, *Azospirillum* cells are remarkably chemotactic [38, 44], aerotactic [2], and redox-tactic [27]. Recently, it was shown that the colonization of soybean and wheat roots as well as the root-to-root motility of *Azospirillum* in agar and sand are active processes that are influenced by attractants [7].

The aims of this study were to elucidate the possible plant host range of *A. brasilense* and to explore further the inter-root motility previously demonstrated in agar and sand under laboratory conditions [7] by investigating this phenomenon with different plant species in soils and under field conditions.

Materials and methods

Bacterial Strains, Growth Conditions, and Plant Inoculation

Two bacterial strains were used in the motility study: *A. brasilense* Cd (ATCC 29710; highly motile strain, mot⁺) and a nonmotile spontaneous mutant (mot⁻) derived from the Tn5 mutant of strain Cd [7] (Strain 29710-10b is described in detail elsewhere [15, 18].) The identical antigenic characteristics of the mot⁻ derivative allowed us to use antibodies raised against strain Cd (mot⁺) for its determination by enzyme-linked immunosorbent assay (ELISA). The mot⁻ mutant was isolated by (a) evaluating its inability to swarm on a solid medium surface [29] and (b) by light microscopy of nonmotile bacteria obtained from the logarithmic phase of growth or from very young colonies (<24 h). The chosen mutant was completely nonmotile during 16 h of continuous analysis by an image analysis system. In root colonization studies, we used strains Cd and Sp-245 [1] and to a lesser extent, strain Cd mot⁻. Bacterial strains were grown in Nutrient Broth (Difco USA).. Bacteria were inoculated onto plants at a concentration of 10⁶ colony-forming units (CFU) per milliliter as previously described [5, 8, 9].

Plants

Wheat plants (*Triticum aestivum*) cv. "Deganit" were used as test species in most experiments and prepared for inoculation as previously described [3, 5]. Plant species and their respective families are summarized in Table 1. Crop plant cultivars used in the colonization study were wheat (*T. aestivum*), Deganit; pearl millet (*Pennisetum americanum*), Gahi 3; sorghum (*Sorghum bicolor*), Savanna 5; corn (*Zea Mays*), Jobilli; tomato (*Lycopersicon esculentum*), Naama; pepper (*Capsicum annum*), Maor; eggplant (*Solanum melongena*), Malka Shechora; cucumber (*Cucumis sativa*), Bet-alfa; melon (*Cucumis melo*), Hemed; canola (*Brassica campestris*), Westar; cotton (*Gossypium barbadense*), Pima S-5; carrot (*Daucus carota* L. var. *sativa*), Tip-top; sugar beet (*Beta vulgaris*), Monnac; soybean (*Glycine max*), Pella; pea (*Pisum sativum*), Laxton Progress; bean (*Phaseolus vulgaris*), Dark red kidney.

Plant Growth Conditions and Field Experiment

Seeds were transferred to plastic growth trays containing soil (20 × 30 × 100 cm; [depth/width/length] containing 20 plants each, 5 cm apart), and maintained under controlled conditions in a growth chamber described previously [3, 5, 6]. The trays were inoculated in a single spot (the inoculated area size was

Table 1. Root colonization of weeds and crop plants from the same botanical families by *A. brasilense* strains Cd (mot⁺, mot⁻) and Sp-245^a

| Weed/crop plant | Botanical family | CFU/g (d. wt) roots | |
|---|------------------|---------------------------|---------------------------|
| | | Cd | Sp-245 |
| Annuals (winter weeds) | | | |
| <i>Isatis aleppica</i> scop. | Brassicaceae | $3.2 \pm 0.7 \times 10^3$ | $4.6 \pm 0.4 \times 10^3$ |
| <i>Sinapsis arvensis</i> L | | $5.1 \pm 0.8 \times 10^3$ | $7.2 \pm 0.6 \times 10^3$ |
| <i>Erucaria myagroides</i> (L) Hal. | | $2.4 \pm 0.5 \times 10^3$ | $3.3 \pm 0.7 \times 10^3$ |
| <i>Brassica nigra</i> (L) Koch. | | $4.7 \pm 0.9 \times 10^3$ | $6.6 \pm 0.4 \times 10^3$ |
| <i>Notobasis syriaca</i> (L) cass. | Asteraceae | $4.9 \pm 0.7 \times 10^4$ | $5.1 \pm 0.3 \times 10^4$ |
| <i>Scolymus maculatus</i> L | | $3.8 \pm 0.1 \times 10^4$ | $4.7 \pm 0.8 \times 10^4$ |
| <i>Chrysanthemum segetum</i> L | | $7.2 \pm 0.6 \times 10^4$ | $1.4 \pm 0.4 \times 10^5$ |
| <i>Chrysanthemum coronarium</i> L | | $9.1 \pm 0.6 \times 10^4$ | $1.1 \pm 0.1 \times 10^5$ |
| <i>Anthemis pseudoctula</i> Boiss. | | $2.9 \pm 0.5 \times 10^4$ | $3.2 \pm 0.7 \times 10^4$ |
| <i>Anthemis melanolepis</i> Boiss. | | $6.3 \pm 0.8 \times 10^4$ | $6.7 \pm 0.5 \times 10^4$ |
| <i>Ormenis mixta</i> (L) DC | | $5.5 \pm 0.1 \times 10^4$ | $8.2 \pm 0.6 \times 10^4$ |
| <i>Cichorium pumilum</i> Jacq. | | $3.8 \pm 0.3 \times 10^4$ | $5.6 \pm 0.5 \times 10^4$ |
| <i>Carthamus tenuis</i> (Boiss.) Bornm. | | $7.8 \pm 1.2 \times 10^4$ | $2.2 \pm 0.8 \times 10^5$ |
| <i>Senecio vulgaris</i> L | | $2.4 \pm 0.5 \times 10^4$ | $2.6 \pm 0.7 \times 10^4$ |
| <i>Senecio vernalis</i> L | | $2.8 \pm 0.7 \times 10^4$ | $3.1 \pm 0.7 \times 10^4$ |
| <i>Ammi visnaga</i> (L) Lam. | Apiaceae | $6.9 \pm 0.7 \times 10^3$ | $1.2 \pm 0.3 \times 10^4$ |
| <i>Daucus aureus</i> Desf. | | $4.1 \pm 0.4 \times 10^4$ | $6.7 \pm 1.4 \times 10^4$ |
| <i>Ridolfia segetum</i> (L) Moris. | | $5.7 \pm 0.9 \times 10^4$ | $8.8 \pm 1.6 \times 10^4$ |
| <i>Stellaria media</i> (L) Vill. | Caryophyllaceae | $7.5 \pm 1.3 \times 10^4$ | $9.6 \pm 1.7 \times 10^4$ |
| <i>Silene gallica</i> L | | $4.8 \pm 0.6 \times 10^4$ | $7.7 \pm 0.6 \times 10^4$ |
| <i>Beta vulgaris</i> L | Chenopodiaceae | $2.1 \pm 0.5 \times 10^5$ | $2.4 \pm 0.7 \times 10^5$ |
| <i>Lavatera trimestris</i> L | Malvaceae | $4.2 \pm 0.8 \times 10^6$ | $5.1 \pm 0.4 \times 10^6$ |
| <i>Malva nicaeensis</i> All. | | $5.5 \pm 1.3 \times 10^6$ | $5.8 \pm 0.3 \times 10^6$ |
| <i>Urtica urens</i> L | Urticaceae | $2.8 \pm 0.7 \times 10^4$ | $3.5 \pm 0.6 \times 10^4$ |
| <i>Urtica pilulifera</i> L | | $5.8 \pm 1.2 \times 10^4$ | $7.5 \pm 1.4 \times 10^4$ |
| <i>Phalaris paradoxa</i> L | Poaceae | $6.2 \pm 0.7 \times 10^5$ | $7.5 \pm 1.2 \times 10^4$ |
| <i>Phalaris brachystachys</i> L | | $4.9 \pm 0.5 \times 10^5$ | $7.3 \pm 1.4 \times 10^5$ |
| <i>Avena sterilis</i> L | | $3.8 \pm 0.9 \times 10^5$ | $4.7 \pm 0.6 \times 10^5$ |
| <i>Ranunculus arvensis</i> L | Ranunculaceae | $6.3 \pm 1.4 \times 10^3$ | $9.1 \pm 0.7 \times 10^3$ |
| <i>Lupinus hirsutus</i> L | Fabaceae | $4.4 \pm 0.4 \times 10^7$ | $4.8 \pm 0.6 \times 10^7$ |
| <i>Medicago ciliaris</i> (L) Krock | | $2.1 \pm 0.7 \times 10^7$ | $2.8 \pm 0.8 \times 10^7$ |
| <i>Vicia vulgare</i> L | | $8.9 \pm 0.8 \times 10^6$ | $1.8 \pm 0.5 \times 10^7$ |
| <i>Papaver rhoeas</i> L | Papaveraceae | $4.8 \pm 0.6 \times 10^4$ | $6.1 \pm 0.5 \times 10^4$ |
| Perennials (winter weeds) | | | |
| <i>Gladiolus segetum</i> Gawl | Iridaceae | $6.8 \pm 0.7 \times 10^4$ | $8.6 \pm 0.7 \times 10^4$ |
| <i>Cynara syriaca</i> Boiss. | Compositae | $4.8 \pm 1.2 \times 10^4$ | $5.2 \pm 0.2 \times 10^4$ |
| <i>Geranium tuberosum</i> L | Geraniaceae | $3.6 \pm 0.6 \times 10^4$ | $6.8 \pm 0.8 \times 10^4$ |
| <i>Polygonum equisetiforme</i> S et S | Polygonaceae | $4.3 \pm 0.6 \times 10^4$ | $7.7 \pm 0.5 \times 10^4$ |
| Annuals (summer weeds) | | | |
| <i>Solanum villosum</i> (L) Lam. | Solanaceae | $2.8 \pm 0.6 \times 10^6$ | $4.7 \pm 0.5 \times 10^6$ |
| <i>Datura stramonium</i> L | | $3.4 \pm 0.2 \times 10^6$ | $5.3 \pm 0.7 \times 10^6$ |

Table 1. (continued)

| Weed/crop plant | Botanical family | CFU/g (d. wt) roots | |
|--|------------------|------------------------------|------------------------------|
| | | Cd | Sp-245 |
| <i>Setaria verticillata</i> (L) BP | Poaceae | $4.8 \pm 0.4 \times 10^5$ | $6.2 \pm 0.7 \times 10^5$ |
| <i>Amaranthus retroflexus</i> L | Amaranthaceae | $6.7 \pm 0.8 \times 10^5$ | $6.9 \pm 0.6 \times 10^5$ |
| <i>Amaranthus graecizans</i> L | | $5.1 \pm 0.6 \times 10^5$ | $6.3 \pm 1.1 \times 10^5$ |
| <i>Chenopodium opulifolium</i> Schrad. | Chenopodiaceae | $3.7 \pm 0.2 \times 10^4$ | $5.7 \pm 0.7 \times 10^4$ |
| Perennial (summer weeds) ^b | | | |
| <i>Cynodon dactylon</i> (L) Pers. | Poaceae | $5.3 \pm 0.4 \times 10^{5c}$ | $5.9 \pm 0.8 \times 10^5$ |
| <i>Sorghum halepense</i> (L) Pers. | | $6.3 \pm 0.8 \times 10^5$ | $7.1 \pm 1.2 \times 10^5$ |
| <i>Convolvulus arvensis</i> L | Convolvulaceae | $5.5 \pm 0.6 \times 10^4$ | $6.9 \pm 1.3 \times 10^4$ |
| <i>Echbalium elaterium</i> (L) Rich. | Cucurbitaceae | $6.8 \pm 1.2 \times 10^4$ | $9.7 \pm 1.4 \times 10^4$ |
| <i>Prosopis forcata</i> Eig | Fabaceae | $3.2 \pm 0.5 \times 10^6$ | $5.9 \pm 1.1 \times 10^6$ |
| Crop plants ^b | | | |
| Wheat | Poaceae | $6.9 \pm 0.8 \times 10^{4c}$ | $4.6 \pm 0.7 \times 10^{4b}$ |
| Pearl millet | | $4.2 \pm 0.5 \times 10^5$ | ND |
| Sorghum | | $5.6 \pm 0.5 \times 10^5$ | $9.6 \pm 0.4 \times 10^4$ |
| Corn | | $2.1 \pm 0.5 \times 10^7$ | $6.9 \pm 0.9 \times 10^6$ |
| Tomato | Solanaceae | $3.6 \pm 0.6 \times 10^6$ | $2.1 \pm 0.8 \times 10^6$ |
| Pepper | | $2.7 \pm 0.2 \times 10^6$ | $7.7 \pm 0.7 \times 10^5$ |
| Eggplant | | $3.4 \pm 1.1 \times 10^6$ | ND |
| Cucumber | Cucurbitaceae | $5.2 \pm 0.4 \times 10^4$ | ND |
| Melon | | $3.4 \pm 0.5 \times 10^4$ | ND |
| Canola | Brassicaceae | $7.6 \pm 0.4 \times 10^3$ | ND |
| Cotton | Malvaceae | $5.6 \pm 0.7 \times 10^6$ | $4.6 \pm 0.3 \times 10^6$ |
| Carrot | Apiaceae | $8.8 \pm 0.7 \times 10^3$ | ND |
| Sugar beet | Chenopodiaceae | $6.5 \pm 1.2 \times 10^4$ | ND |
| Soybean | Fabaceae | $4.2 \pm 0.2 \times 10^7$ | $3.3 \pm 0.6 \times 10^7$ |
| Pea | | $6.8 \pm 0.6 \times 10^7$ | $4.8 \pm 1.2 \times 10^7$ |
| Bean | | $2.3 \pm 0.5 \times 10^7$ | ND |

Plant's nomenclature according to Cohen [20] and Zohary [45].

^aCFU, colony-forming units; d. wt, dry weight; ND, not determined.

^bStrain Cd mot⁻ was determined only for several crop plants.

^cStrain Cd mot⁺

5 × 5 × 1 cm [w/l/d]) by beads inoculant carrier as described later for field inoculation and were maintained under saturated water-holding capacity by adding sterile deionized water throughout the experiments.

In the field, seeds were hand-sown in a light-textured sandy soil (Haploxeralfs) (soil characteristics are described below), 20 cm apart in plots of 2 × 2 m using a grid. Inoculation was carried out during sowing with dry alginate-bead inoculant [4]; six beads were manually placed with a homemade applicator around each seed. Field experiments were treated under a commercial agrotechnical regimen but without the application of herbicides [14]. Irrigation was applied periodically by above-the-foliage sprinklers every 2–3 days to maintain moisture conditions at 100% of water-holding capacity. In experiments designed to maintain saturated soil conditions, the irrigation was controlled by a drip system using an irrigation computer programmed to maintain saturated soil conditions. Experiments using soil columns were performed as previously described [11] using undisturbed soil core samples [19] taken from the field and transferred into glass columns in the laboratory. The soil columns were connected to a water reservoir, which permitted continuous percolation of water throughout the columns.

Table 2. Technical details of the experiments

| Plant species and experimental setup | Environment | Soil type |
|---|--|----------------------------|
| Colonization of 64 plant species | Pots in temperature-controlled greenhouse | Artificial soil mixture |
| Movement between wheat plants | Trays with soil in controlled growth chamber | Sandy soil |
| Movement between wheat plants and various weeds | Trays with soil in controlled growth chamber | Sandy soil |
| Movement between wheat plants and various weeds | Field conditions | Sandy soil |
| Survival of bacteria in wheat rhizosphere | Field conditions | Sandy soil |
| Movement in soil without plants | Soil columns in controlled growth chamber | Three light-textured soils |
| Movement in soil without plants | Field condition | Sandy soil |

Root colonization studies were done with plants growing in 500 ml of commercial black plastic pots containing 450 ml of artificial soil mixture consisting of peat/vermiculite/sand (1:1:1, v/v). Crop plant seeds were disinfected as previously described [3, 5] before inoculation with bacterial suspension [5], but weed seeds or reproductive organs were similarly inoculated without disinfection because of a lack of data on these propagules' response to the disinfectant. The plants were grown for 30–45 days after inoculation in a temperature-controlled greenhouse ($22 \pm 3^\circ\text{C}$ for winter plants and $28 \pm 3^\circ\text{C}$ for summer plants). The root bacteria population was measured as described later. All technical details of each experiment are summarized in Table 2.

Soils

In most experiments, we used light-textured, sandy soil (Haploxeralfs) with a low water-holding capacity of 8.6% (v/v), organic matter content of 1.3%, clay content of 4.3%, and pH of 8.1. The soil was sterilized in large glass containers by tyndallization (three times, 1 h each time at 24-h intervals). The plastic trays were disinfected with 10% commercial hypochlorite and later thoroughly rinsed with sterile distilled water before loading with the sterile soil. Two additional light-texture soils, Gypsiorthids and Torriorthents, were also used, having the following characteristics: (a) Gypsiorthids: water-holding capacity of 14.4% (v/v), organic matter content of 0.4%, clay content of 20.1%, and pH of 7.4; (b) Torriorthents: water-holding capacity of 10.1% (v/v), organic matter content of 0.4%, clay content of 12.3%, and pH of 8.0.

Quantification of Bacteria on Roots

Bacteria from plants growing in artificial soil were counted as follows: The entire plant was removed from the pot, and all loose "soil mixture" particles were shaken out. The roots were rinsed in sterile deionized water until no visible soil particles could be observed. Bacteria were identified and counted by the indirect-ELISA method, which is highly specific for strain Cd and its derivative [30, 31], or combined with the limited enrichment method [16] when the number of bacteria was lower than 10^4 CFU per milliliter (the lower limit of our ELISA method). In natural soils, *A. brasilense* CFU were

counted by conventional plate count on nutrient agar (Difco) after incubation of 48–72 h at $30 \pm 1^\circ\text{C}$, using the typical colony morphology of strain Cd as a marker (dry ridges in dark pink colonies) [9].

Experimental Design and Statistical Analysis

All indoor experiments were done in triplicate (one tray or soil column as a replicate), and each was repeated two to three times. Field experiments were carried out in 4 m^2 plots in which only the center ($1 \times 1 \text{ m}$) was analyzed, leaving 1 m as a buffer zone around each analyzed plot. Each experiment was conducted in five plots. The number of bacteria per root was obtained from all plots (triplicate sampling per plot), pots and trays, and analyzed by one-way analysis of variance (ANOVA) at $P \leq .05$.

Results

Azospirillum brasilense Strains Cd and Sp-245: Root Colonization of Weeds and Crop Plants From the Same Botanical Families

Root colonization by two strains of *A. brasilense* (Cd and Sp-245) on greenhouse seedlings of 64 plant species belonging to 19 different botanical families revealed that the bacteria were capable of colonizing all plant species of its “common” population level (Table 1, and compare to data in [13]). Different levels of colonization were detected among the botanical families, the highest being for Malvaceae, Fabaceae and Solanaceae, and the lowest for Brassicaceae and Apiaceae. However, both strains were capable of colonizing weeds and crop plants belonging to the same botanical family and at similar population levels. In general, strain Sp-245 was a slightly better colonizer than strain Cd 45 days after inoculation (Table 1). Inoculation of several crop plants with the nonmotile strain (Cd mot⁻) resulted in root colonization similar to the parental strain (Cd).

Movement of A. brasilense Mot⁺ and A. brasilense Mot⁻ From Inoculated Wheat Roots to Noninoculated Wheat Roots

In soil trays, *A. brasilense* mot⁺ migrated from inoculated roots to the noninoculated roots of the adjacent plants, within 32 days after inoculation. The colonization level of the adjacent, noninoculated roots was similar to that of the original inoculated root but decreased as the distance from the inoculated plants increased (Table 3).

Although the water-holding capacity was similar in the trays with both mot⁺ and mot⁻ strains of *A. brasilense*, a different pattern was detected in the mot⁻ strain. The inoculated plants were colonized; however, bacteria did not move from the inoculated roots to the adjacent, noninoculated roots (Table 3).

Survival of A. brasilense Mot⁺ and A. brasilense Mot⁻ in Wheat Plants in the Field

Both bacterial strains survived well in the rhizosphere of the inoculated wheat plants for a period of 30 days. The population first decreased, then increased slightly (Fig. 1). In the case of the motile wild-type strain, all the adjacent plants became colonized. However, in experiments using the nonmotile mutant, all adja-

Table 3. Movement of *A. brasilense* mot⁺ and *A. brasilense* mot⁻ from inoculated roots to noninoculated roots in the soil 32 days after inoculation

| Bacterial strain | Plant location | No. of <i>A. brasilense</i> /g (fresh wt. roots) |
|------------------|---|--|
| Mot ⁺ | Inoculated plant | $5.2 \pm 1.4 \times 10^5$ a ^e |
| | Adjacent plant (5 cm) | $4.4 \pm 1.3 \times 10^5$ a |
| | Plants growing 30 cm from inoculated plants | $2.7 \pm 0.9 \times 10^4$ b |
| | Plants growing 50 cm from inoculated plants | $6.3 \pm 0.7 \times 10^3$ c |
| Mot ⁻ | Inoculated plant | $3.7 \pm 1.1 \times 10^4$ b |
| | Adjacent plant (5 cm) | 0 |
| | Plants 30 cm from inoculated plants | 0 |
| | Plants 50 cm from inoculated plants | 0 |

^eDifferent letters indicate significant difference at $P \leq 0.05$ by ANOVA

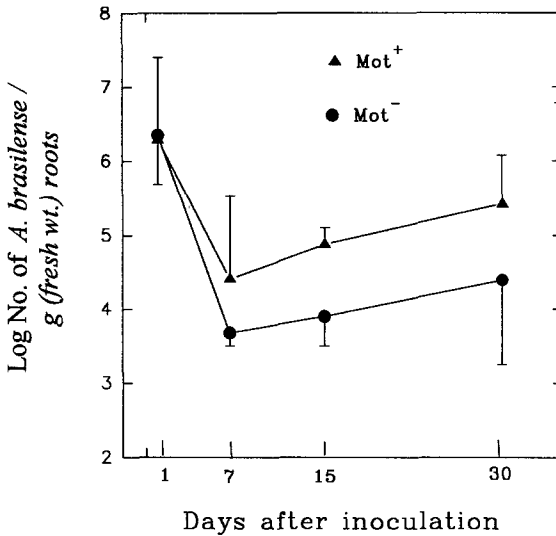


Fig. 1. Survival of *A. brasilense* mot⁺ and *A. brasilense* mot⁻ in the wheat rhizosphere in the field. Bars represent SD.

cent plants, except one, remained uncolonized even if the soil was periodically saturated by irrigation (data not shown).

Movement of A. brasilense Mot⁺ and A. brasilense Mot⁻ From Inoculated Wheat Plants to Adjacent Weeds in the Field

Because the experimental wheat field was not treated with herbicides, many weeds emerged randomly and grew beside the inoculated wheat plants. Analysis of each weed plant revealed that all the weed roots were colonized by *A. brasilense* mot⁺ but not by *A. brasilense* mot⁻ (Fig. 2). The colonization level of the roots of five different weed species was similar to that of wheat roots (Table 4).

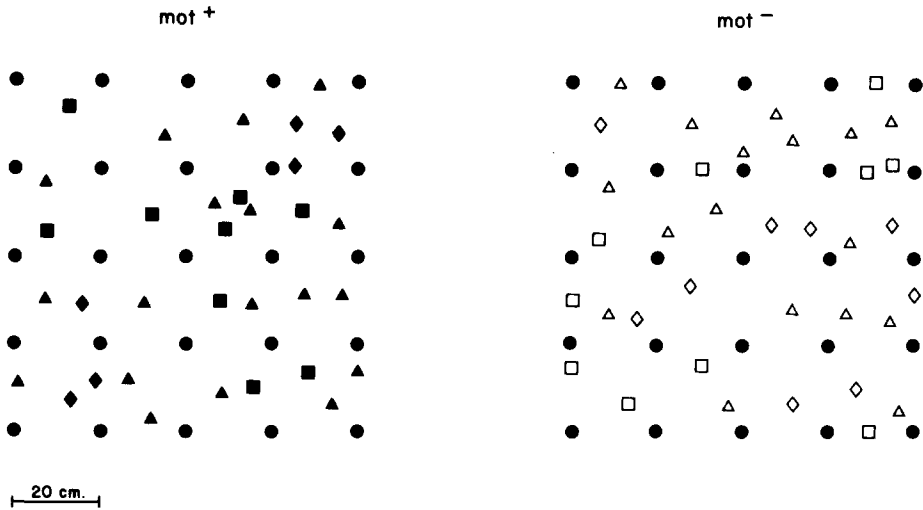


Fig. 2. Movement of *A. brasilense* mot⁺ and *A. brasilense* mot⁻ from inoculated wheat plants to weeds in the field. Solid symbols represent colonized plants, and open symbols represent uncolonized plants. *Phalaris paradoxa* + *Phalaris brachystachys* (mixed populations) (▲); *Malva aegyptia* (■); *Notobasis syriaca* + *Silybum marianum* (mixed populations) (◆); wheat (●).

Table 4. Movement of *A. brasilense* mot⁺ and *A. brasilense* mot⁻ from inoculated wheat plants to weeds in the field

| Bacterial strain | Weed species | No. of <i>A. brasilense</i> lg (fresh wt. roots) in noninoculated weeds | |
|---------------------------------------|---|---|---|
| Mot ⁺ | <i>Phalaris paradoxa</i> plus <i>Phalaris brachystachys</i> ^a | 4.2 ± 1.9 × 10 ⁵ a ^b | |
| | <i>Malva aegyptia</i> | 2.7 ± 1.6 × 10 ⁵ a | |
| | <i>Notobasis syriaca</i> plus <i>Silybum marianum</i> ^a | 4.1 ± 0.4 × 10 ⁵ a | |
| | Wheat | 3.8 ± 1.2 × 10 ⁵ a | |
| | Mot ⁻ | <i>Phalaris paradoxa</i> ⁺ | 0 |
| | | <i>Phalaris brachystachys</i> ^a | 0 |
| <i>Malva aegyptia</i> | | 0 | |
| <i>Notobasis syriaca</i> ⁺ | | 0 | |
| <i>Silybum marianum</i> ^a | | 0 | |
| Wheat | | 6.8 ± 0.8 × 10 ⁴ | |

^aMixed plant population (seedlings of the two species are morphologically similar at this stage of growth).

^bThe same letter indicates no significant difference at $P \leq .05$ by one-way analysis of variance.

Movement of *A. brasilense* Mot⁺ Among Weed Species

A. brasilense mot⁺ moved from inoculated weeds to noninoculated weeds growing in soil trays regardless of their botanical species or whether they were annuals or perennials (Table 5). The colonization level of the recipient plants depended on

Table 5. Movement of *A. brasilense* mot⁺ between weed species of different botanical families, annuals, and perennials growing in soil trays^a

| Donor plant | Recipient plant | No. of <i>A. brasilense</i> g (fresh wt. root) in recipient plant |
|-------------------------------------|-------------------------------------|---|
| <i>Chrysanthemum coronarium</i> (A) | <i>Brassica nigra</i> (A) | 6.4 ± 0.6 × 10 ³ |
| <i>Chrysanthemum coronarium</i> (A) | <i>Beta vulgaris</i> (A) | 3.4 ± 0.8 × 10 ³ |
| <i>Avena sterilis</i> (A) | <i>Chrysanthemum coronarium</i> (A) | 1.2 ± 0.4 × 10 ⁵ |
| <i>Lupinus hirsutus</i> (A) | <i>Avena sterilis</i> (A) | 5.4 ± 1.1 × 10 ⁵ |
| <i>Avena sterilis</i> (A) | <i>Geranium tuberosum</i> (P) | 4.8 ± 0.9 × 10 ⁴ |
| <i>Geranium tuberosum</i> (P) | <i>Avena sterilis</i> (A) | 4.1 ± 0.7 × 10 ⁵ |
| <i>Cynodon dactylon</i> (P) | <i>Amaranthus retroflexus</i> (A) | 3.6 ± 0.3 × 10 ⁵ |
| <i>Amaranthus retroflexus</i> (A) | <i>Cynodon dactylon</i> (P) | 8.4 ± 0.9 × 10 ⁵ |
| <i>Cynodon dactylon</i> (P) | <i>Daucus aureus</i> (A) | 9.4 ± 1.2 × 10 ³ |
| <i>Daucus aureus</i> (A) | <i>Cynodon dactylon</i> (P) | 6.3 ± 0.6 × 10 ⁵ |

^a A, annual; P, perennial.

Means ± SE (n = 6).

the botanical family; some botanical families appeared to be more favorable hosts (see this also in Table 1).

Movement of A. brasilense Mot⁺ and *A. brasilense* Mot⁻ in Water-Saturated Soil in the Absence of Plants

Water-saturated soil columns of three different soils were inoculated with the mot⁺ and mot⁻ strains. Both strains remained in the upper layer of the column and did not migrate downward with the percolating water (Fig. 3A). No difference between the three soil types or the two bacterial species was recorded (data not shown). In the water-saturated soil of the field, the bacteria released from the bead inoculant were detected just below the soil crust (1–5 mm depth) (Fig. 3B).

Discussion

Root colonization by beneficial bacteria (active or passive attachment to the root surface of inoculated bacteria) may be fundamental to increased plant productivity [13, 35]. *Azospirillum* spp. are known to be efficient colonizers in diverse environments. This well-studied plant growth-promoting rhizobacterium (PGPR) has the potential to increase the yield of many crop plants by directly affecting plant growth through yet-to-be-revealed mechanisms [13].

The pioneer inoculants of *Azospirillum* are currently in the commercial market despite inconsistent evidence of their effectiveness [34]. For application purposes, *Azospirillum* cells are commonly incorporated into various types of inoculant carriers, which, because of their agrotechnical nature, are unable to ensure that the bacteria will encounter the emerging root (14,24). Thus, bacterial movement from the inoculation site to the root site is essential if root colonization is to occur. This movement, from a few micrometers to several centimeters, must occur in an

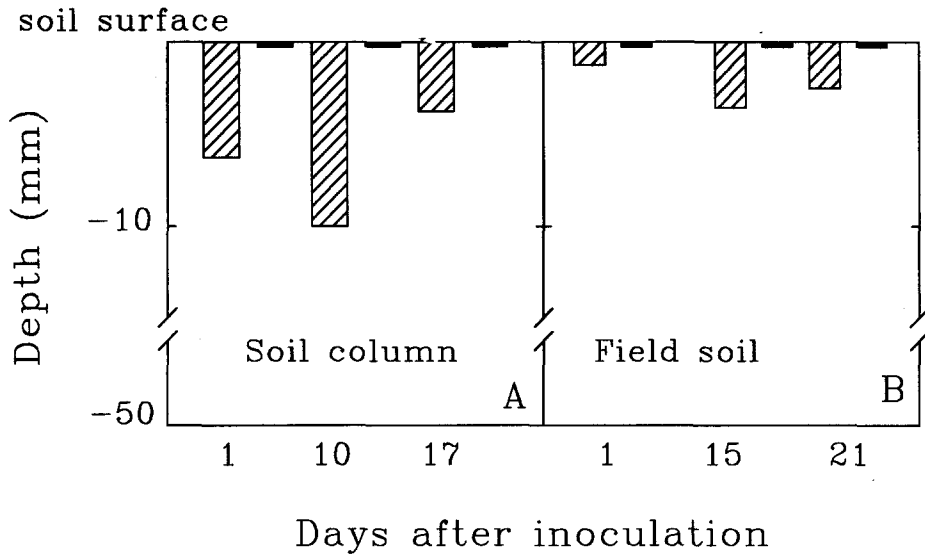


Fig. 3. Map of downward movement of *A. brasilense* mot⁺ in water-saturated sandy soil in an undisturbed soil column (A) and in the field (B) in the absence of plants. Bacterial location with percolating water (▨); bacterial location without percolating water (—). Minus symbol in the Y axis symbolizes depth below soil surface.

environment of fierce competition with other soil flora, which are also seeking nutrients and root colonization sites on the growing seedlings [3]. Thus, self-motility of PGPR can be considered an important trait for rhizobacteria [33].

Passive dispersion by percolating water, especially in semi-arid conditions that lack sufficient water [where *Azospirillum* showed its best performance (39)], cannot explain how *Azospirillum* colonizes the entire root system. Therefore, it is plausible that bacterial motility is responsible for this dispersion. A previous study indicated that *A. brasilense* can move from wheat to soybean and vice versa in soft agar and in water-saturated sand [7]. Here, we extended our study to the movement of *Azospirillum* in the soil to emphasize the importance of motility in root colonization both in crop and weed plants.

This study revealed that *A. brasilense* motility in soil is essential to colonization of the root system. Although the nonmotile mutants proliferated on the inoculated roots similarly to the wild type, they failed to colonize neighboring roots, even though water for passive transport was available. Under our tray experimental conditions, the rhizospheres were almost certainly confluent, because roots of adjacent plants created a uniform root mass indistinguishable from one plant to the other. This facilitated the movement of beneficial bacteria between adjacent plants. Therefore, we assume that the “soil-free” distances in which *A. brasilense* moved in our study might be measured in millimeters rather than centimeters, although the bacterium can migrate about 30 cm in “root-free” soil [7, 10]. Our results with *A. brasilense* give support to studies that show that nonmotile mutants of beneficial biocontrol pseudomonads were impaired in their ability to move toward seeds [40] or colonize roots [23] when compared to their wild-type parent strains.

Motility of wild-type strains in the rhizosphere appears to increase the survival probabilities of *Azospirillum*. *Azospirillum* is totally dependent on the presence of roots to survive because it survives poorly in some soil types [11] and does not move downward in the plantless field with percolating water [10]. The soil surface is a dangerous location for bacteria as it dries. However, when the bacterial cells have the ability to colonize different plant species, as demonstrated here, the bacterium can migrate to neighboring plants if the original host plant dies.

A broad range of hosts is an obvious advantage for any given beneficial bacterium, eliminating the need for developing many specific crop-bacterial combinations, which are confusing to growers, especially those in less developed countries. The full host range of *Azospirillum* has not previously been defined. Claims of *Azospirillum* specificity for certain cereals species are documented [for review see 13]. The evidence presented here, however, shows otherwise. Under controlled conditions, the bacteria colonized the root systems of 64 plant species belonging to 19 different botanical families. It had no preference for crop plants or weeds, or for annuals or perennials. This list supported previous studies showing *Azospirillum*'s ability to colonize both wild and crop plants worldwide [13, 32, 36]. It seems that *Azospirillum* is a general root colonizer. This fact, as satisfactory as it might be for the inoculation industry, raises some points of caution. In the inoculated field, the growth of the local weeds also might be enhanced [13]. Furthermore, most studies on the host range of *Azospirillum* have been conducted on a limited scale (greenhouse, axenic conditions, and/or controlled conditions etc.); therefore, these findings should be verified under field conditions before any definite conclusions are drawn.

It is proposed (a) that *A. brasilense* is not a plant-specific bacterium; (b) that bacterial motility within the plant root system and between neighboring plants is the mechanism responsible for bacterial dispersion, which leads to the colonization of the entire root system and adjacent plants; and (c) that *A. brasilense* dispersion in soil depends on the presence of plants.

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