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Wheat Root Tips as a Vector for Passive Vertical Transfer of *Azospirillum brasilense* Cd

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The ability of wheat root tips to serve as efficient vertical vectors for passive transfer of *Azospirillum brasilense* Cd was evaluated in sterilized growth chambers containing agar, sand or soil. Most root tips, whether from main or lateral roots, were colonized by *A. brasilense* Cd and were capable of transferring this bacterium to a depth of 290 mm from the inoculation site. The location of *A. brasilense* Cd was directly dependent on root tip location; whenever root tips passed through a bacterial layer, regardless of depth, they became inoculated. However, when root tips failed to reach the inoculation site they were not colonized by *A. brasilense* Cd. Seed inoculation resulted in an even distribution of *A. brasilense* Cd along the entire root system. Inoculation at various depths in the growth medium resulted in an uneven bacterial distribution and bacteria were concentrated mainly in the elongation and small root-hair zones as well as in the inoculation site. *A. brasilense* Cd multiplied on the root tip during its vertical movement. However, bacterial movement in the root vicinity was minimal. *A. brasilense* Cd did not spread from the root surface to the rhizosphere when exogenous nutrients were supplied in this area, but did so in the presence of chemoattractants. It is suggested that the prevalence of *A. brasilense* Cd deeper than the initial inoculation site is a result of their passive transfer by the growing root tip.

INTRODUCTION

Beneficial rhizosphere bacteria of the genus *Azospirillum* are usually applied to the soil or onto the seeds of cereals and vegetables in order to colonize seedling roots and to improve plant growth (Bashan *et al.*, 1989*a*; Okon, 1985; Patriquin *et al.*, 1983). Monitoring the distribution of *Azospirillum* cells on the roots of many cereals has shown that they are scattered unevenly along the roots (Albrecht *et al.*, 1983; Döbereiner & Day, 1976; Okon & Kapulnik, 1986; Patriquin & Döbereiner, 1978; Reinhold *et al.*, 1986). In wheat roots, *A. brasilense* Cd cells have been found as deep as 50 cm from the initial inoculation site on the soil surface (Bashan & Levanony, 1987; Bashan *et al.*, 1987; Bashan & Wolowelsky, 1987). However, it remains uncertain how the cells moved through the soil layers from the inoculation site.

Azospirillum strains have significant chemotactic ability both *in vitro* (Barak *et al.*, 1983; Heinrich & Hess, 1985; Mandimba *et al.*, 1986; Reinhold *et al.*, 1985) and in the soil (Bashan, 1986*c*; Bashan & Levanony, 1988*b*). However, chemotaxis may not fully explain vertical movement of bacterial cells on the root surface. In addition, *Azospirillum* cells attach to soil particles (Bashan & Levanony, 1988*c*, *d*) and to root surfaces (Gafni *et al.*, 1986) by a network of fibrillar material which is strong enough to prevent their random movement from the attachment sites (Bashan *et al.*, 1986; Bashan & Levanony, 1988*a*; Okon & Kapulnik, 1986).

The aims of this study were to: (i) determine if *A. brasilense* Cd cells attached to the root tips of wheat are transferred vertically; (ii) evaluate whether this transfer is active or passive on the

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Abbreviation: TTC, 2,3,5-triphenyltetrazolium chloride.

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part of the bacterium; (iii) determine if bacteria are dispersed evenly on the root surface; and (iv) find out if there is a local migration of bacteria on the root surface.

METHODS

Plant growth conditions. Wheat seedlings (*Triticum aestivum*) cv. 'Deganit' were surface-disinfected with 1% NaOCl for 5 min and then thoroughly washed with sterile water. Seeds were imbibed for 5 h in water prior to transfer to the agar growth chamber. The growth chambers (Fig. 1) consisted of two glass plates (20 × 20 cm) held apart by three PVC sticks (5 mm thick). Each cell was assembled with six clips. The assemblage was alcohol flame-sterilized and sealed with 3–5 ml of sterile 1% (w/v) water agar at 40 °C. Soft agar (0·5%) was added until the entire space between the glass plates was filled. After cooling, disinfected seeds were placed on top of the agar and the growth chamber was sealed with Parafilm (replaced daily to avoid oxygen deficiency) to prevent desiccation. The growth chamber was wrapped with aluminium foil to avoid direct illumination of the developing root system and transferred to a fully controlled growth chamber (Conviron, model EF7H, Controlled Environments Co., Canada). Experiments employing quartz sand or soil were done in similar growth chambers (20 × 30 cm) made of plexiglass.

Bacterial growth conditions. Azospirillum brasilense Cd (ATCC 29710) was grown in nutrient broth (Difco) liquid medium, in Erlenmeyer flasks equipped with shallow grooves in a rotary shaker (250 r.p.m., 14–16 h, 30 ± 2 °C, exponential phase of growth). This procedure improved culture aeration and prevented the aggregation of *A. brasilense* Cd, thus enabling a culture yield of single cells after relatively short growth periods. Bacteria were prepared for plant inoculation as previously described (Bashan, 1986*a*; Bashan & Levanony, 1985).

Bacterial inoculation. Disinfected wheat seeds were inoculated by one of the following methods. (i) Seeds were imbibed for 1 h at ambient temperature under a weak vacuum created by a small water pump to facilitate penetration of double-washed *A. brasilense* Cd (10^6 or 10^8 c.f.u. ml^{-1}) into the seed cavities. Inoculated seeds were then transferred to the plant growth chambers. (ii) The construction of the agar growth chamber enabled inoculation of root tips at any depth required. *A. brasilense* Cd (10^8 c.f.u. ml^{-1}) was mixed (1 : 1, v/v) with 1% sterile agar solution (38 ± 1 °C), and a layer (1–2 mm thick) was spread on the solidified 0·5% agar layer at various depths inside the growth chamber. After solidification of the bacteria/agar mixture, the rest of the growth chamber was filled with 0·5% agar solution and wheat seeds were placed on its upper surface as described above. *A. brasilense* Cd was tested for viability after being embedded in the agar for 7 d (which is longer than the duration of the experiments). Roots became inoculated with *A. brasilense* Cd when they grew through the embedded bacterial layer. The chemoattractants glycine or aspartic acid (10 mM), or the nutrients KNO_3 (10 mM), sodium malate (100 mM), sodium lactate (100 mM) or sodium succinate (100 mM), were embedded in a similar fashion to that used for the bacterial culture at a site 50 mm below the seed. For experiments on transfer of *A. brasilense* Cd by lateral roots, root tips from main roots were removed by small surgical scissors after removal of one glass plate. The plate was then returned. To avoid contamination of non-inoculated plants by plate removal, the control plants were grown in an identical growth chamber which remained intact throughout the experiment. Inoculation of sand or soil (quartz sand and Crosby silt loam soil, Columbus, Ohio, USA; 40% clay) in growth chambers was done by partially filling the chamber with moist (field capacity) soil or sand (sterilized by tyndallization, three times for 1 h for each sterilization, soil containers being incubated at ambient temperature between sterilizations) to the required depth. A solidified layer of 0·5% agar supplemented with 5×10^8 c.f.u. of *A. brasilense* Cd was then carefully placed on the soil surface and the chamber was filled with soil or sand.

Optimization of the performance of the agar growth chamber. The following experimental variables were tested: agar concentration (0·5, 0·75 and 1%, w/v); 2,3,5-triphenyltetrazolium chloride (TTC) concentration (0·05, 0·1, 0·15 and 0·25%, w/v); light intensity (60, 130 and 200 $\mu E\ m^{-2}\ s^{-1}$); Parafilm-sealed versus open systems; and various temperatures (constant 15 °C; 25 ± 2 °C; and 18 °C night, 22 °C day). Experiments could be done successfully using each combination of variables listed above. However, the selected optimal conditions were: 0·5% agar, 0·15% TTC, light intensity of 130 $\mu E\ m^{-2}\ s^{-1}$, Parafilm-sealed cells and temperatures of 18 °C (night, 14 h) and 22 °C (day, 10 h). All experiments described herein were done under these conditions.

Detection and enumeration of bacteria on roots. Bacterial colonies on the root surface were visibly detected by using the TTC-reducing method of Patriquin & Döbereiner (1978). At the end of the plant growth period, one of the glass plates was removed and a 0·15% solution of TTC in 0·06 M-potassium phosphate buffer, pH 6·8, supplemented with 0·15 M-NaCl (PBS), was added dropwise onto the root surfaces. The glass plate was replaced and the growth chambers were returned to the incubator. Development of pink colour zones (detected and measured using a scaled stereoscopic microscope, Nikon, Japan) after 3–4 h indicated large masses of bacteria reducing the colourless TTC (Smith, 1951). Non-inoculated wheat roots produced pink zones only after 24 h incubation.

Direct detection of *A. brasilense* Cd in the soil was impossible due to the opacity of the soil. Therefore, *A. brasilense* Cd was detected by carefully removing roots from the sand or soil, slightly rinsing them in tap water and carefully spreading them using a glass rod in shallow plexiglass trays. Minimal amounts of liquid containing TTC were spread over the roots, and the trays were covered with transparent plastic film and incubated for 3 h.

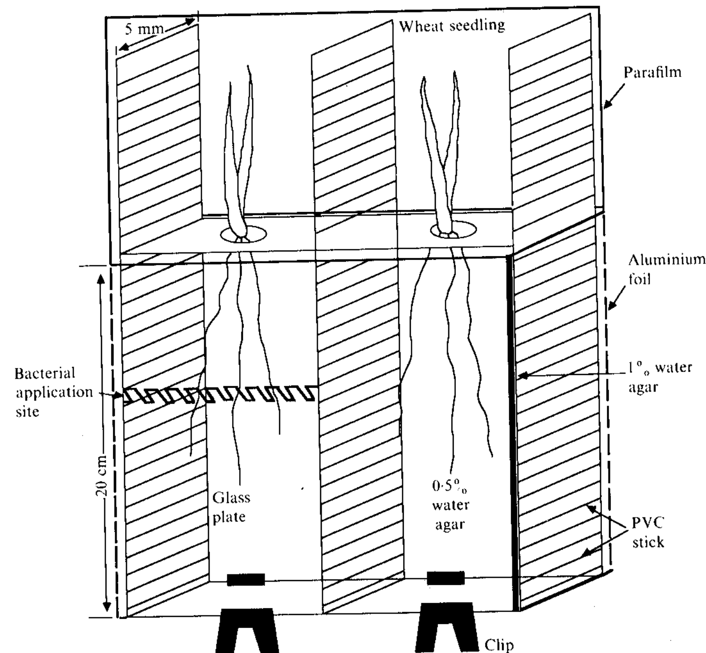

Fig. 1. Schematic representation of the plant growth chamber used for determining bacterial transfer by root tips. Note that, for clarity, the vertical and horizontal dimensions are not to the same scale.

Development of pink bands was scored and measured as in the agar chamber. The presence of bacteria was verified by cutting both pink and colourless sections of the roots, homogenizing the sections, and counting the bacteria by the improved selection technique of Bashan & Levanony (1985). Bacteria were identified by an ELISA method (Levanony *et al.*, 1987). Bacterial counts on the root tips were carried out as follows. Root tips (2 mm long) were excised with a razor blade and placed in 2·5 ml PBS. Cells of *A. brasilense* Cd attached to the outer surface of the root tips were released into the buffer by sonication of the sample in a Branson sonifier for 4 min at 50% power. This sonication did not affect the viability of *A. brasilense* Cd (unpublished data). The suspension was plated on nitrogen-free medium (Bashan & Levanony, 1985) using a spiral plater (Spiral Systems, Cincinnati, Ohio, USA); the plates were incubated for 72 h at 30 ± 2 °C and colonies counted.

Measurement of transfer of A. brasilense Cd by root tips. The distance of bacterial transfer by the root tip was determined by measuring the distance of the pink bands from the inoculation site (whether from the seed site or from inoculation at various depths).

Experimental design and statistical analysis. All experiments were done in triplicate (one growth chamber as a replicate) and each was repeated two to four times. Each measurement reported is the mean of three to five root tips. Significance is given as standard error (SE).

RESULTS

Rate of transfer of A. brasilense Cd by wheat roots

Following seed inoculation, the presence of the furthermost bacterial band in relation to the root tip position was recorded. Bacteria were detected in close proximity to the root tips, and most root tips (> 98% of those evaluated) contained bacterial microcolonies. The faster the roots grew into the agar (root growth rates of 3·43 mm h^{-1} were recorded 48–96 h after inoculation under these conditions), the deeper the bacteria were detected. Bacterial cells or bands did not randomly migrate through the very soft agar from the root surface (Fig. 2).

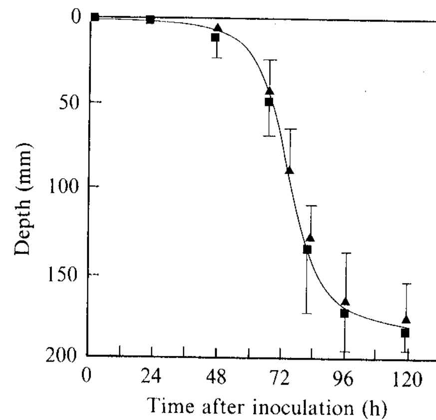

Fig. 2. Rate of transfer of *A. brasilense* Cd by wheat roots. ■, Root tip position; ▲, bacterial presence near the root tip. Each point represents the mean of six to nine separate determinations of root tips. Experiments were repeated twice. Bars represent SE.

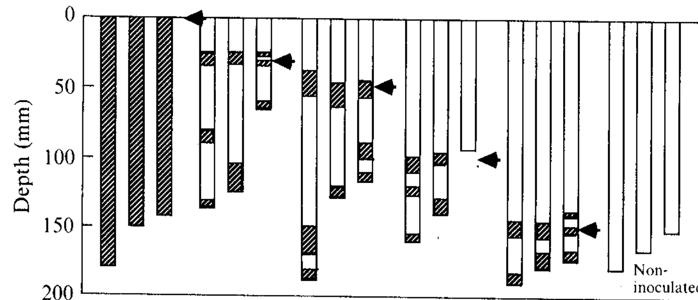

Fig. 3. Transfer of *A. brasilense* Cd by root tips inoculated at various depths. Each group of three columns shows the lengths of the three main roots of one wheat seedling. Hatched areas represent the sites and sizes (in mm) of pink bands on the roots. Zero indicates the seed site and the arrows show the site at which *A. brasilense* Cd was embedded in the agar. The experiment was repeated three times; the results are from one representative experiment.

Table 1. *Multiplication of A. brasilense Cd on the surface of the root tip during vertical transfer by the root tip in agar-filled plant growth chambers*

Inoculum (c.f.u. ml^{-1})	Depth of bacterial inoculation site (mm)	Distance of root tip from inoculation site (mm)		No of *A. brasilense* Cd (c.f.u. per root tip)‡	
		After 24 h	After 72 h	After 24 h	After 72 h
10^3	0*	22 ± 4	146 ± 15	$4 \pm 0{\cdot}3 \times 10^2$	$3{\cdot}3 \pm 0{\cdot}3 \times 10^3$
10^6				$3 \pm 0{\cdot}2 \times 10^3$	$3{\cdot}2 \pm 0{\cdot}2 \times 10^3$
10^3	0†	21 ± 5	149 ± 23	$4{\cdot}1 \pm 0{\cdot}4 \times 10^2$	$2{\cdot}8 \pm 0{\cdot}4 \times 10^3$
10^6				$2{\cdot}7 \pm 0{\cdot}3 \times 10^3$	$2{\cdot}9 \pm 0{\cdot}3 \times 10^3$
10^3	−50	18 ± 3	80 ± 14	$2{\cdot}8 \pm 0{\cdot}4 \times 10^2$	$2{\cdot}1 \pm 0{\cdot}3 \times 10^3$
10^6				$2{\cdot}4 \pm 0{\cdot}2 \times 10^3$	$2{\cdot}7 \pm 0{\cdot}4 \times 10^3$
10^3	−100	12 ± 4	32 ± 9	$4{\cdot}1 \pm 0{\cdot}5 \times 10^2$	$2{\cdot}3 \pm 0{\cdot}4 \times 10^3$
10^6				$2{\cdot}1 \pm 0{\cdot}3 \times 10^3$	$2{\cdot}6 \pm 0{\cdot}2 \times 10^3$

* Seed inoculation.
† Surface inoculation.
‡ Counted on BL semi-selective medium (Bashan & Levanony, 1985).

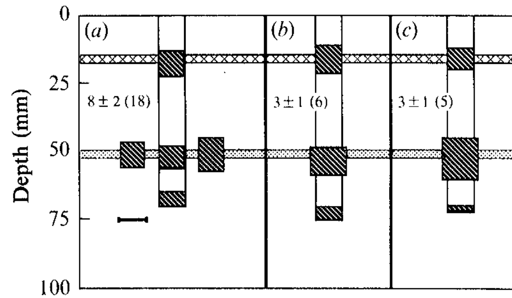

Fig. 4. Transfer of *A. brasilense* Cd by root tips in the presence of the chemoattractant glycine (*a*) and the nutrients KNO_3 (*b*) and malate (*c*). Each column represents the largest root of one wheat seedling. ⊠, Site at which bacteria were embedded in the agar; ▨, attractant or nutrient site. Hatched areas represent the site and sizes (in mm) of pink bands on the roots or in the surrounding agar. Zero indicates seed site. The horizontal bar represents 5 mm. Numbers followed by SE are the mean distance of the band (in mm) from the root surface measured in 24 to 32 different roots. Numbers in parentheses are the distance (mm) for the furthermost band found in all experiments. Experiments with attractants were repeated three times and those with nutrients were repeated twice. Data are shown from one representative experiment.

Transfer of A. brasilense Cd by root tips inoculated at various depths

When a root tip passed through a bacterial layer it became colonized by *A. brasilense* Cd. Seed inoculation resulted in distribution of *A. brasilense* Cd along the entire root system. Inoculation of root tips at any depth (up to 150 mm beneath the seed site) resulted in colonization of *A. brasilense* Cd in the root tip area. Distribution of *A. brasilense* Cd along the roots was non-homogeneous and concentrated mainly in the zone of elongation and in the small root-hair zone. Upward movement of *A. brasilense* Cd from the inoculation site was negligible and bacterial cells did not migrate into the water agar surrounding the roots. When root tips failed to reach the inoculation site, they were not colonized by *A. brasilense* Cd (Fig. 3).

Quantitative analysis of the total number of *A. brasilense* Cd in the pink bands revealed that the bacteria multiplied on the root tips (Table 1). The initial population was dependent on the inoculum concentration; at a low cell density (10^3 c.f.u. ml^{-1}), the number of *A. brasilense* Cd on the root tip ranged from 3×10^2 to 4×10^2 c.f.u. per root tip 24 h after inoculation. Multiplication of this initial population resulted in a population density of $2{\cdot}1 \times 10^3$ to $3{\cdot}3 \times 10^3$ c.f.u. per root tip after 72 h. When a denser inoculum (10^6 c.f.u. ml^{-1}) was used, only slight multiplication was observed with time (Table 1). These detected bacterial numbers correspond to 5×10^3 to 2×10^6 c.f.u. (g root fresh wt)$^{-1}$. *A. brasilense* Cd was found in low numbers (10^1 to 5×10^2 c.f.u. (g root fresh wt)$^{-1}$ in most non-coloured zones. No *A. brasilense* Cd cells were found in non-inoculated wheat seedlings.

Transfer of A. brasilense Cd by root tips in the presence of chemoattractants or nutrients

Wheat root tips were inoculated by embedded *A. brasilense* Cd 15 mm below the seed site and the attractants or nutrients were embedded 50 mm below the seed site. After the root tips passed these sites, the presence of *A. brasilense* Cd was examined along the roots and in the surrounding agar. In all cases *A. brasilense* Cd was found on roots at the inoculation site as well as on every root tip. With the chemoattractant glycine, two defined bacterial bands migrated from the root surface towards the attractant. The furthermost band was detected 18 mm away from the root. Since the attractant had diffused through the agar layer, the bacterial bands were larger than at the site of the original position of the attachment (Fig. 4*a*). Similar results were obtained with aspartic acid (data not shown). Embedding of nutrients known to be consumed by *A. brasilense* Cd such as KNO_3 or sodium malate resulted in only slight movement of *A. brasilense* Cd away from the root surfaces. In no case were separate bands formed (Fig. 4*b*, *c*). Similar results were obtained with the other nutrients (sodium lactate and sodium succinate; data not shown).

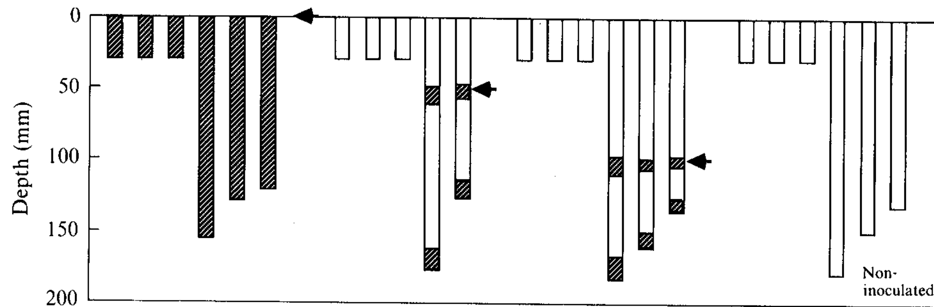

Fig. 5. Transfer of *A. brasilense* Cd by root tips of lateral roots. The groups of five or six columns represent the size of all the roots of one wheat seedling. Hatched areas represent the sites and sizes (in mm) of pink bands on the roots. Zero indicates the seed site and arrows the site at which *A. brasilense* Cd was embedded in the agar. The experiment was repeated three times; the results are from one representative experiment.

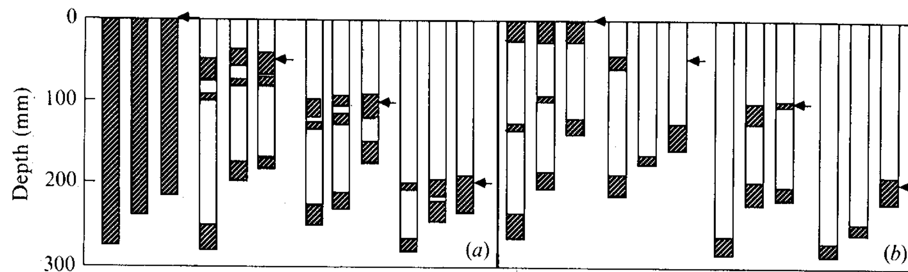

Fig. 6. Transfer of *A. brasilense* Cd by root tips grown in quartz sand (*a*) and in soil (*b*). Hatched areas represent the sites and sizes (in mm) of pink bands on the roots. Zero indicates seed site and arrows the site at which *A. brasilense* Cd was embedded in the sand or soil. The sand experiment was repeated twice and the soil experiment three times. Results are from a representative experiment.

Transfer of A. brasilense Cd by root tips of lateral roots

The tips of the main roots were excised and transfer of *A. brasilense* Cd by the developing lateral roots was measured. Generally, tips of lateral roots were as efficient as those of main roots in transfer of *A. brasilense* Cd deeper than the inoculation site. *A. brasilense* Cd colonies were found in all root tips tested as well as close to the inoculation zone, whereas upward migration along the root to the seed site was minimal (Fig. 5).

Transfer of A. brasilense Cd by root tips grown in sand and soil

Transfer of *A. brasilense* Cd by roots grown in sand resembled that observed in agar (Fig. 6*a*). However, since the growth chambers were larger, *A. brasilense* Cd was located as deep as 280–290 mm from the soil surface, irrespective of the depth of inoculation. Seed inoculation resulted in equal colonization along the entire root surface. As in the agar growth chamber, no significant upward migration of *A. brasilense* Cd to the seed site was detected even if the sand was kept under field capacity conditions throughout the experiment. The trend of transfer of *A. brasilense* Cd by root tips grown in soil was similar to that in sand (Fig. 6*b*). All root tips were colonized by *A. brasilense* Cd. However, possibly as a result of the more intensive washing required to clean roots from soil particles, fewer bands were detected and even seed inoculation did not result in complete coverage of the root surface by *A. brasilense* Cd in soil. As in sand, root tips colonized by *A. brasilense* Cd were detected as deep as 290 mm below the soil surface. The size of *A. brasilense* Cd populations in sand or soil was very similar to the bacterial populations detected in agar chambers.

DISCUSSION

The question of how *Azospirillum* cells colonize plant roots in deep soil layers when inoculated onto the soil surface or into the seed bed may have several explanations. (i) Bacterial cells may be washed down either by rain or by irrigation into the root system (Okon, 1985); (ii) *Azospirillum* cells may reach the roots by motility towards a possible exudate(s) secreted by the growing seedling (Bashan, 1986*c*); (iii) bacteria may be transferred by soil vectors, e.g. microfauna, insects or worms (Bashan & Levanony, 1987); or (iv) passive downward transport of bacteria may be mediated by the growing root tips. Downward movement of *Azospirillum* in water streams seems unlikely since nearly all the bacteria are adsorbed to soil particles immediately upon application (Bashan & Levanony, 1988*c*, *d*). Bacterial motility in the soil is slow and is restricted to very small distances (Bashan, 1986*c*), thus preventing the bacterium from reaching its target site on the root. Fauna vectors capable of efficient transfer of *Azospirillum* have not yet been identified. Therefore, this study focused on the evaluation of the role of the growing root tip in vertical transfer of *Azospirillum* into deep soil layers.

The tetrazolium staining procedure described by Patriquin & Döbereiner (1978) for observing *Azospirillum* cells on cereal roots, combined with immunological (ELISA; Levanony *et al.*, 1987) and conventional bacterial counts, gave a reliable method for precise detection of bacterial location along the roots.

Colonization of roots by *Azospirillum* is known to be non-homogeneous, and bacterial cells have been observed throughout the entire root system of many plant species. However, it has been established that this bacterial genus has some preference for the zones of elongation and for the bases of root-hairs (Bashan *et al.*, 1986; Okon & Kapulnik, 1986; Patriquin *et al.*, 1983) as well as for the root tip zone (Levanony *et al.*, 1989; Murty & Ladha, 1987; Nishizawa *et al.*, 1983), in which the earliest effects of inoculation, i.e. enhancement of cell division and enlargement of the elongation zone, are observed (Levanony & Bashan, 1989). These zones provide nutrients that may enhance the metabolically active association (Heulin *et al.*, 1987; Nishizawa *et al.*, 1983) and may attract *Azospirillum* from the rhizosphere (Mandimba *et al.*, 1986). Therefore, it is logical that *Azospirillum*-colonized root tips, upon penetrating the soil layers, may carry down the bacterial cells. This transfer involves only the surface population of *Azospirillum*, since there are no intercellular spaces in the root tip. However, it is possible that the internal *Azospirillum* population located in the intercellular spaces of the elongation zone (Levanony *et al.*, 1989) is transferred by the same mechanism. Furthermore, this study demonstrates that multiplication of *Azospirillum* on the root tips occurs. Thus, even a few cells on the root tip may, in time, create a substantial population. These data are not in accordance with those of Okon & Kapulnik (1986), who did not detect multiplication of the same *Azospirillum* species on the root tip.

Many studies have indicated that the optimal level for successful inoculation with *Azospirillum* is 10^5–10^6 c.f.u. ml^{-1} (Bashan, 1986*a*; Bashan *et al.*, 1989*a*, *b*; Kapulnik *et al.*, 1985; Okon, 1985), and this is also the bacterial population found per g fresh weight of roots. Below this threshold no effects are detected on plant growth. The present study showed that inoculation levels (10^3 c.f.u. ml^{-1}) lower than the optimum can, in time, produce the same final bacterial population on the roots. Therefore, it can be proposed that the main effects of *Azospirillum* on plants occur immediately upon inoculation and that a relatively large amount of inoculated bacteria is required since the bacterial population developed later, even if relatively large, has little effect.

All *Azospirillum* species are microaerophilic (Barak *et al.*, 1982; Hurek *et al.*, 1987; Nelson & Knowles, 1978; Nur *et al.*, 1982). The colonization of root tips is of advantage for these cells because, when the roots penetrate deeper into the soil layer, the oxygen supply is lower and competition with the aerobic bacterial population of the rhizosphere is therefore reduced. However, analysis of *Azospirillum* sites along the roots revealed that they are found particularly on young roots, and much less frequently on the older parts of the roots (Bashan & Levanony, 1987). In contrast, preferential establishment of *A. lipoferum* was observed within the upper parts of sorghum roots (Baldani *et al.*, 1986), and this was interpreted as a requirement of the bacteria for medium-aged roots with many laterals, which favour infection. This view is not in

agreement with many studies which have demonstrated that the most marked effects of inoculation are in young plants (Albrecht *et al.*, 1981; Bashan, 1986*a*; Sarig *et al.*, 1984; Smith *et al.*, 1984). However, our study gave support to the role of lateral roots (Baldani *et al.*, 1986) by demonstrating that lateral root tips are as efficient as main root tips in transferring *Azospirillum* cells. Therefore, it can be concluded that in plants which develop a highly branching root system, the probability of successful colonization by *Azospirillum* is high.

Azospirillum species have a substantial chemotactic ability both *in vitro* (Barak *et al.*, 1983; Heinrich & Hess, 1985; Reinhold *et al.*, 1985) and in soil under a controlled environment (Bashan 1986*c*). However, the present study casts some doubt on the basic hypothesis of the inoculation technology, which claims that if bacteria are applied in close proximity to the roots, they will reach the roots by chemotaxis. We present evidence that the root tip only became colonized when it reached the bacteria. *Azospirillum* did not migrate towards the root. Nevertheless, it appears that attractants have a major effect on bacterial behaviour in the rhizosphere. For example, application of exogenous attractants into the rhizosphere of colonized roots resulted in migration of cells away from the root to the rhizosphere, and this also occurred when exogenous nutrients were supplied. Therefore, it appears that the exact role of chemotaxis *in vivo* in the soil is not obvious and needs more detailed studies.

The growth rate of wheat roots is extremely high in moist soil, and friction with soil particles may remove the attached bacteria. It is known that upon colonizing root surfaces *Azospirillum* cells anchor by a network of fibrillar material (Bashan *et al.*, 1986; Okon & Kapulnik, 1986). The results presented here give further evidence that the attachment is strong enough to keep the bacterial cells on the root surface. However, this binding prevents any movement of *Azospirillum* cells along the root surface. Thus, secondary dissemination of *A. brasilense* Cd in the root system is very restricted and depends mainly on root growth rather than bacterial motility.

Two methods of *Azospirillum* inoculation are frequently used: seed inoculation and soil inoculation using various bacterial carriers (Okon, 1985). The data presented here indicate that seed inoculation is more efficient than inoculation on or into the soil, although, by both methods, *Azospirillum* cells are transferred efficiently by the root tips to a similar depth. In the case of seed inoculation, the entire root system is colonized by *Azospirillum* whereas in soil inoculation, colonization is fragmented. However, soil inoculation can be used in cases when seed inoculation is impossible if the proper inoculant carrier is used (Bashan, 1986*b*), since *A. brasilense* Cd survives poorly in soil (Albrecht *et al.*, 1983; Bashan & Levanony, 1988*c*).

In conclusion, this study suggests that *Azospirillum* cells detected in deep roots of wheat (Bashan & Levanony, 1987) were transferred passively to these sites by the growing root tips.

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