MICROBIAL ECOLOGY

Airborne Transmission of the Rhizosphere Bacterium *Azospirillum*

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Abstract. In controlled environments, plants inoculated with *Azospirillum brasilense* caused the contamination of noninoculated plants via air transmission. This was detected up to 6 m from the inoculation source. In the temperate agricultural zone studied in field experiments, local *Azospirillum* strains were detected year-round. Other diazotrophs showed a similar distribution pattern. It is proposed that (1) contamination from *Azospirillum*-inoculated plants may occur via airborne bacteria, (2) local azospirilla and other diazotrophs have an airborne phase in temperate agricultural zones, and (3) because of the existence of an airborne phase for Gram-negative rhizosphere bacteria, inoculation presents a risk of uncontrolled airborne contamination.

Introduction

Bacterial strains belonging to the genus *Azospirillum* are present in plant rhizospheres in diverse geographical regions worldwide and are frequently isolated from tropical, semi-arid, arid, and temperate zones [13, 16, 19, 22, 27, 30]. Diazotrophs are present in nearly every soil, regardless of climatic conditions [2, 14, 23, 24, 261.

When *Azospirillum* is inoculated into the plant rhizosphere in pot experiments, many experimental designs take into consideration the possibility of contamination of control plants by *Azospirillum* cells transferred by excess water drainage from inoculated pots. Various agrotechnical measures have been designed in order to counteract this undesirable contamination [10]. Nevertheless, random sampling of noninoculated plants revealed that some plants contained the inoculated strain in their roots despite all precautions. The source of this contamination remains unknown.

It is known that Gram-negative, phyllosphere, phytopathogenic bacteria are dispersed among geographical regions, and from field to field, by various biotic vectors and abiotic vehicles [3]. Such dispersal of rhizosphere *Azospirillum*

strains, except by artificial inoculation, is not documented. Also, the ability of *Azospirillum* to survive in the air like sewage bacteria [12] has not yet been reported and has been considered negligible or nonexistent since the genus *Azospirillum* does not contain spores.

The objectives of the present study were: (1) to define whether *Azospirillum* has an airborne capability; (2) to detect possible movement of airborne *Azospirillum* from inoculated to noninoculated plants under controlled conditions, and (3) to find out if *Azospirillum* and other diazotrophs can be isolated from the air over farmland areas of a temperate zone.

Materials and Methods

Bacteria

Azospirillum brasilense Cd (ATCC 29710) was used as the inoculant strain. Local *A. brasilense* and *A. lipoferum* were characterized according to Tarrand et al. [29].

Bacterial Growth Media

The nitrogen-free medium defined by Döbereiner and Day [15] as modified by Bashan and Levanony (BL) [5] was used for the recovery of bacteria from the air. On this medium, *A. brasilense* Cd colonies appeared as a distinctive typical pink color after 5-8 days of incubation [11, 18]. Other azospirilla and diazotrophs showed various colors ranging from colorless to white and yellow. Nutrient agar (Difco Laboratories, USA) was used for growing *A. brasilense* Cd.

Acetylene Reduction Assay (ARA)

ARA was performed as described by Hardy et al. [21] on pure cultures and was expressed as nmole C_2H_4/tube/24 hours.

Plant Growth Conditions and Inoculation

Soybean (*Glycine max* cv. Pella) plants were grown in 800 ml pots (5 seedlings/pot) containing autoclaved peat: vermiculite: loam soil (1:1:1; v/v/v) in a temperature-controlled greenhouse (8 x 6 x 4 m) at $25 \pm 3°C$ (day) and $20 \pm 3°C$ (night) under natural illumination. Prior to planting, seeds were disinfected (5% NaOCl for 30 min with shaking at 50 rpm) and thoroughly washed with sterile tap water. At the age of first true leaf (5-7 days after sowing), the seedlings were inoculated with double-washed [0.06 M potassium phosphate buffer, pH 7.0, supplemented with 0.15 NaCl (PBS)] exponential-phase cells of *A. brasilense* Cd at a concentration of 5×10^8 Cfu/ ml, and were grown as previously described [5]. Inoculation was performed in the greenhouse by irrigating 50 ml bacterial suspension onto the "soil" surface. In one treatment, a layer of 4 cm-deep sterile fine vermiculite was carefully spread on top of the inoculated soil surface. This layer remained dry throughout the subsequent air sampling period. In this treatment, inoculation was carried out aseptically in the laboratory, and the pots containing surface vermiculite were later transferred to the greenhouse. Pots were placed on an iron-net table to avoid possible contamination from drainage. Plants were also similarly grown in a growth chamber (4 x 1.5 x 2 m; $25 \pm 3°C$; 16 hours light, 400 $\mu E_{in}/m^2$/sec; Environmental Growth Chamber Co., OH, USA).

Dispersal of A. brasilense *Cd in the Air*

The bacteria were dispersed into the air of the greenhouse and the growth chamber by one of the following methods: (1) 0.1 ml suspension of *A. brasilense* Cd was spread, using a glass rod, on solid nutrient agar medium in five Petri dishes and incubated at $30 \pm 2°C$ for 48 hours. Later, the dish covers were removed and the bacteria that had grown on the agar surface were exposed in the greenhouse for up to 24 hours. A longer exposure time was impossible due to the drying of the agar layer under greenhouse conditions. (2) 20 ml of an exponential-growth-phase *A. brasilense* Cd culture diluted with PBS to 10^9 cfu/ml (1.05 OD_{540}) were aerosolized randomly into the air using an atomizer (this sprayer produced very small droplets and is commonly used for thin layer chromatography sprays). Alternatively, a piece of gauze (10 x 10 cm) was soaked with the culture and was hung in front of the ventilation system. (3) A stationary-growth-phase suspension of *A. brasilense* Cd was aerosolized as above after the bacterial aggregates that had formed in this growth stage were disintegrated by a mild sonication (10 W, 3 min, Branson Sonic Power Co., CT, USA). This sonication is harmless to *A. brasilense* Cd [7]. (4) "Soil" mixture (100 ml) was inoculated with 20 ml of 5 x 10^8 cfu/ml, spread on a flat Plexiglas tray (20 x 5 x 1 cm), and placed in front of the ventilation system.

Sampling the Air Bacterial Content in Controlled Environments and in Open Fields

Gravity Deposition Method from the Greenhouse and Growth Chamber Air

Samples were collected from the four corners of a greenhouse and from its center by exposing 9 cm (in diameter) Petri dishes containing N-free medium (described above) for 4 hours. Trap dishes were mounted horizontally and vertically, 120 cm above the greenhouse floor (one dish per position, sampling site, and time, for a total of 10 dishes per sampling). The greenhouse and the growth chamber had a horizontal ventilation system (air current speed of 2-4 m/sec) and controlled temperature of $25 \pm 3°C$ during sampling time (day). After exposure, lids were replaced and the dishes were sealed with parafilm and incubated at $25 \pm 1°C$ for 4 days. Developing colonies were sampled and transferred twice in succession to a fresh solid Nfree medium. The original dishes were then incubated at $30 \pm 1°C$ for an additional 3 days to detect *A. brasilense* Cd. The typical pink colonies of *A. brasilense* Cd were treated as above. These colonies were further identified as *A. brasilense* Cd according to the descriptions of this strain [11, 18]. The air dispersal distance was measured by placing the inoculum source as close as possible to the ventilation system. *A. brasilense* Cd was trapped 6 m (greenhouse) and 1.5 m (growth chamber) away from the inoculum source, as described above.

Volumetric Method in the Greenhouse and in the Growth Chamber

A four-stage Andersen's volumetric sampler was employed. Each stage contained a plate perforated with 400 holes that was directly under a Petri dish containing N-free medium described above. Air was drawn through the sampler for 2 hours with a small vacuum pump at a rate of 1 x 10^4 cm³/min (total sampling of 1,200 liters). Other details of the system were as described by Andersen [1]. The number of *Azospirillum* and other diazotrophs was calculated from the four stages of the sampler.

Gravity Deposition Method in the Field

Sampling procedure was identical to the gravity method performed in the greenhouse but included an additional dish (in an upside-down position) at every location for a total of 15 dishes per

sampling. Exposure time was 8-9 hours per sampling, starting at 9 a.m. Dishes were attached to a wooden frame, 100 cm above the ground. The sampling area (50 x 50 m) was planted during the sampling period (except in winter) with noninoculated maize, soybean, and other unidentified weeds. Wind speed was calm, varied among sampling periods, and was within the range of 10 to 25 m/hour. After exposure to air, samples were treated as described above, but incubation temperatures of the dishes were $20 \pm 2°C$ (for winter samples) and $30 \pm 2°C$ (for samples in other seasons).

Gravity Deposition Method by a Moving Trap

Five Petri dishes were mounted vertically on the roof of a car with the agar surface facing the drive direction. Car speed varied continuously within the range of 60 to 100 km/hour. Dishes were opened in farmland away from the urban areas. Usually, the agar surface was contaminated with dust particles trapped from the air, which did not prevent later multiplication of the bacteria. In order to assure that the colonies were nitrogen-fixers and not heterotrophic bacteria that derived nitrogen from the dust contamination, the colonies were transferred twice onto fresh N-free medium. Only colonies capable of developing on the third plate series were considered possible diazotrophs and were further analyzed by ARA.

  Colonies from all sources were transferred into 0.5 ml sterile glycerol in microtubes and were kept at -70°C [28] for 10-12 months until characterized. *Azospirillum* strains were isolated from plant rhizospheres as previously described [5].

Bacterial Enrichment from Roots

Enrichment of *A. brasilense* Cd, obtained from the plant roots, was carried out as previously described [5].

Meteorological Data

Meteorological data was obtained from the Statistics Laboratory, Department of Agricultural Engineering, OARDC, Wooster, Ohio, USA.

Sampling Design and Statistical Analysis

Airborne bacteria, especially in the field sampling, was characterized as being variable; dishes contained from many to few colonies. In addition, *Azospirillum* and other diazotrophs were often present in the air in relatively very low numbers. Therefore, data were combined from all the trapped dishes (5 to 15 different dishes/sampling) and analyzed together. Significance is given by $P \leq 0.05$.

Results

Trapping of Airborne A. brasilense *Cd and Diazotrophs in Controlled Environments*

Placing the inoculum source close to the ventilation system of the growth chamber or the greenhouse resulted in air dispersal of bacteria via air currents

Table 1. Occurrence of *A. brasilense* Cd in the air of a growth chamber and greenhouse

Inoculum source	Number of *A. brasilense* Cd trapped (cfu/64 cm^2 medium surface)[d]	
	Growth chamber[e]	Green-house[f]
Petri dishes[a]	52.6 b[g]	32.2 b
Gauze soaked with bacteria[b]	84.4 a	69.6 a
Inoculated "soil"[c]	9.4 c	3.8 c

[a] 318 cm^2 solid nutrient agar surface covered with bacteria
[b] 10 x 10 cm gauze soaked in 109 cfu/ml bacterial suspension
[c] Peat: vermiculite: loam soil (1:1:1; v/v/v) inoculated with 5 x 10^8 cfu/ml substrate
[d] Trapped during 4 hours exposure; area per one Petri dish
[e] 1.5 m distance between the inoculum source and the traps; total air volume of 12 m^3
[f] 6 m distance between inoculum source and traps; total air volume of 192 m^3
[g] Numbers in each column (separately) followed by a different letter differ significantly at $P \leq 0.05$

in these environments. *A. brasilense* Cd was detected as far as 1.5 m (growth chamber) and 6 m (greenhouse) from the inoculum source. The efficiency of the sources varied; gauze soaked with bacteria was the most efficient in releasing *A. brasilense* Cd into the air, whereas inoculated soil was the least efficient (Table 1).

In order to confirm the trapping efficiency of the gravity deposition method, a volumetric air-sampler was employed in the same experiments, both in the growth chamber and in the greenhouse. Both methods were used in experiments that employed gauze soaked with bacteria as the inoculum source. Generally, the volumetric results were confirmed, and had similar trends with regard to bacterial counts obtained by the gravity method (Fig. 1A, B). Despite this, the gravity deposition method was chosen as the preferred method for the entire study because it was simpler to perform. Although accurate, the volumetric sampler method was abandoned because only one unit was available. In addition, the sampler was dependent on a car battery for its air pump. The battery tended to fade after several hours of operation, reducing the air volume that passed through the sampler. These drawbacks make it impractical for use in remote fields.

Presence of inoculated soybean plants for 10 days in the greenhouse resulted in detection of airborne *A. brasilense* Cd. Only a single colony was detected in a greenhouse room free of inoculated plants for 30 days prior to sampling. Covering the inoculated "soil" surface with a thick layer of sterile dry vermiculite prevented air dispersal of *A. brasilense* Cd. Exposure of the greenhouse air to either (1) open Petri dishes containing *A. brasilense* Cd colonies or (2) exponential-phase bacterial cells sprayed into the greenhouse air resulted in detection
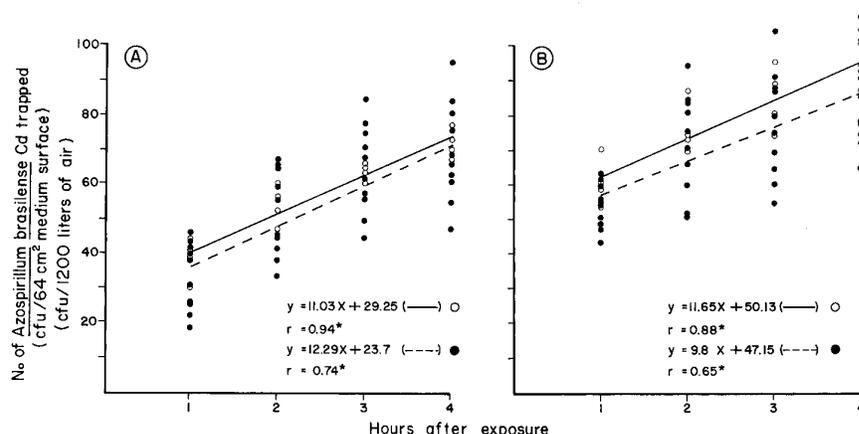
Fig. 1. Linear regression analyses of *A. brasilense* Cd cfu trapped from the air using the gravity (⎯⎯)and the volumetric (-----) methods in the growth chamber (A) and in the greenhouse (B).● : Bacterial counts obtained by the gravity method on a single dish; O: data from volumetric counts. An asterisk represents significance of a regression line at $P \leq 0.05$. Each point represents a single counting by one of the techniques.

of airborne *A. brasilense* Cd cells. This population disappeared 5 days later. Air spraying of stationary-phase bacteria resulted in improved detection in the air compared to the two previous bacterial dispersal treatments. The number of other diazotrophs in the greenhouse air was small, but constant, regardless of *Azospirillum* inoculation treatments (Table 2).

Contamination of Noninoculated Soybean Plants from A. brasilense-
Inoculated Plants Grown Simultaneously in Either a
Growth Chamber or in a Greenhouse

When inoculated and noninoculated soybean plants were simultaneously grown in a growth chamber, most of the noninoculated plants were contaminated regardless of the distance between the two groups. However, contamination of noninoculated plants in the greenhouse was lower and the percentage of contaminated plants decreased as the distance from the inoculated plants increased (Table 3).

Trapping of Azospirillum *and Diazotrophs from the Field*
Air of a Temperate Zone

The numbers of *A. brasilense* isolated from the air of a noninoculated field were similar throughout the warmer seasons of the year, even though two sampling days were relatively cold (3-6 cfu/954 cm^2 medium surface/8 hours).

Table 2. Trapping of *A. brasilense* Cd and diazotrophs from the air
of a controlled greenhouse ($25 \pm 3°C$; 192 m^2)

Greenhouse treatment	No. of *A. brasilense* Cd[a]	No. of diazo- trophs[b]
	(cfu/64 cm^2 medium surface/ 4 hours)	
Presence of 25 inoculated pots containing plants for 10 days prior to sampling	7.2 c[c]	0.3 a
Absence of inoculated plants for 30 days prior to sampling	0.1 d	0.4 a
Presence of 20 inoculated pots covered with 4 cm layer of dry vermiculite on "soil" surface for 6 days prior to sampling	0 d	0.6 a
Exposure of solid medium (5 Petri dishes) covered with *A. brasilense* Cd colonies for one day and sampling after		
1 Day	6.1 c	0.3 a
3 Days	0.4 d	0.5 a
5 Days	0 d	0.3 a
Spraying of 20 ml 10^9 cfu/ml (exponential phase) in the air and sampling after		
1 Day	17.2 b	0.2 a
3 Days	1.1 d	0.3 a
5 Days	0 d	0.4 a
Spraying of 20 ml 10^9 cfu/ml (stationary phase) in the air and sampling after		
1 Day	35.4 a	0.3 a
3 Days	2.8 c	0.6 a
5 Days	1.3 d	0.4 a

[a]Identification according to Eskew et al. [18] and Bashan et al. [11]
of all colonies
[b]Acetylene reduction assays according to Hardy et al. [21] and growth
on N-free medium
[c] Numbers followed by a different letter in each column (separately)
differ significantly at $P \leq 0.05$

This species was detected only once in the winter (2 cfu). Trapping of *A. lipoferum* isolates was lower throughout the year (1-3 cfu). Other diazotrophs followed a similar distribution pattern, but their total number was higher (3-8 and 11-26 cfu, in winter and the rest of the year, respectively) (Table 4). ARA were performed to confirm the presence of nitrogenase in these bacteria (Table 4). Partial characterization of the diazotrophs revealed that they belonged to the following genera: *Bacillus, Clostridium, Pseudomonas, Azotobacter, Klebsiella,* and *Erwinia.* These genera were randomly detected among the different sampling times. Isolation of *Azospirillum* strains from the maize rhizosphere grown in the same field indicates that both *A. brasilense* and *A. lipoferum* were present in seven root and soil samples taken during the entire maize growth season (see also [9]).

Table 3. Contamination of noninoculated soybean plants with *A. brasilense* Cd from inoculated plants grown simultaneously in a growth chamber and in a greenhouse. Plants were analyzed 26 days after inoculation

	Distance between inoculated and non-inoculated plants (m)	No. of inoculated pots		No. of contaminated pots[a]		Total percentage of contamination
		Exp.1	Exp.2	Exp.1	Exp.2	
Growth chamber	none[b]	15	7	12[c]	4	72.7
	0.5	14	6	11	4	75
	1	15	7	13	5	81.8
Greenhouse	none	15	12	9	6	55.5
	1	15	12	4	4	29.6
	1.5	15	11	5	4	34.6
	4	13	12	2	3	20

[a]Number of control pots was equal to the inoculated pots
[b]Inoculated and noninoculated pots were randomly mixed; approximately 10 cm distance between pots
[c]Detection by liquid enrichment method and identification of *A. brasilense* Cd according to[11, 18]

Table 4. Trapping of *Azospirillum* and other diazotrophs from the field air using stationary "trap plates"

Sampling date	Local season	Wind Speed maximum m/hour[a]	Precipitation rain/snow (R, S) mm[a]	Air temp (°C)a	Total no. of *A. brasilense* (cfu/954 cm^2 medium surface)[b]	*A. lipoferum*	Acetylene reduction (nmole C_2H_4/ tube/24 hours)[c]	Total no.of diazotrophs	Acetylene reduction (nmole C_2H_4/ tube/24 hours)[c]
March 23, 1988	Winter	20	0	15	2	0	520	8	940b
Dec. 20, 1988		25	R; 0.2	11	0	0	ND[d]	3	860b
Feb. 3, 1989		15	S; 2	-5	0	0	ND	4	730b
April 24, 1988	Spring	19	0	7	6	2	920c[c]	17	1,350a
May 10, 1988		15	R; 0.3	16	4	3	1,090c	13	1,550a
August 12, 1988	Summer	10	0	27	3	1	1,800a	22	1,750a
August 30, 1988		12	0	15	5	0	1,850a	26	1,620a
Sept. 30, 1988	Autumn	10	R; 0.08	19	4	1	1,620b	16	1,400a
Oct. 24, 1988		12	R; 0.2	3	6	1	1,290c	11	1,330a

[a]According to the Statistics Laboratory, Department of Agricultural Engineering, OARDC, Wooster, Ohio, USA
[b]According to Tarrand et al. [29]
[c]According to Hardy et al. [21]
[d]ND: not determined
[e]Numbers in each column followed by a different letter differ significantly at $P \leq 0.05$

Table 5. Trapping airborne *Azospirillum* and diazotrophs in the farmland of central Ohio using "trap plates" mounted on a moving automobile

Road no.	Sampling location From	To	Dist. (km)	Sampling date	Air temp. (°C)[b]	Total no. of A. brasilense	A. lipoferum (cfu/318 cm² medium surface)[c]	Acetylene reduction (nmole C_2H_4/24 tube/24 hours)[d]	Total no. of diazotrophs	Acetylene reduction (nmole C_2H_4/tube/24 hours)[d]
SR-62[e]	Wooster	New Albany	150	Jan. 27, 1989	1[g]	0[a]	0[a]	ND[i]	4[a]	80b
SR-83[e]	Wooster	(Columbus)		May 5, 1989	9	8	6	850 b[h]	28	1,100a
SR-3[e]	Wooster	Mt. Vernon	70	Apr. 28, 1989	10	6	3	910 b	22	1,300a
SR-95[e]	Wooster	Mt. Gilead	90	June 1, 1988	25	5	1	1,850 a	17	1,220a
				Sept. 4, 1988	18	7	4	2,100 a	11	1,460a
I-71[f]	Columbus	Mansfield	90	June 12, 1988	22	1[a]	0[a]	2,660	3[a]	1,430a
I-70[f]	Columbus	Zanesville	70	Aug. 28, 1988	19	0	0	ND	5	1,520a

[a] Per 254 cm²
[b] Average temperature according to the Statistic Laboratory, Department of Agricultural Engineering, OARDC, Wooster, Ohio, USA
[c] According to Tarrand et al. [29]
[d] According to Hardy et al. [21]
[e] Secondary highways, 2 traffic lines, light car traffic during sampling
[f] Interstate roads, major highways, 6–8 traffic lines, heavy car traffic during sampling
[g] The medium in the plates froze as a result of chill factor; plates were thawed immediately after in the laboratory
[h] Numbers in each column followed by a different letter differ significantly at $P \leq 0.05$
[i] ND: not determined

Trapping Azospirillum *and Diazotrophs from the Countryside*
Air by a Moving Trap

A. brasilense was trapped from the farmland air of central Ohio by moving traps from spring until autumn (5-8 cfu/318 cm^2 surface medium). Trapping of *Azospirillum* was possible on secondary, low-traffic country roads and impossible on the major, heavily trafficked highways crossing the same farmland areas. Trapping of *A. lipoferum* followed the trapping pattern of *A. brasilense* (1-6 cfu). Neither *Azospirillum* species was trapped during a winter sampling, taken under low temperatures and snow cover (Jan. 27, 1989). Higher numbers of other diazotrophs (confirmed by ARA) were trapped on the farmland roads (11-28 cfu), whereas lower numbers of diazotrophs were trapped on the major highways and in the winter sampling (Table 5). The diazotroph genera detected resembled those located in the field and were described above. *A. brasilense* and *A. lipoferum* were also isolated from six samples taken from roots and soil of several unidentified wild grasses grown alongside the country roads (SR-62, SR-83, and SR-3) near Wooster, Ohio, in August, 1988.

Discussion

Strains of the rhizosphere bacterium *Azospirillum* are frequently isolated and later inoculated into the plant rhizosphere in order to improve plant growth and productivity [8]. Neither the presence of *Azospirillum* in the air nor its natural transport by air currents from one field to another or between different geographical regions has been previously studied.

The presence of *Azospirillum* in the air, as demonstrated in this study, suggests the possibility of a transport mechanism for this organism across larger distances than the known root-to-root movement [6] or limited self-motility in the soil [4]. The airborne *Azospirillum* cells probably became airborne from the surface soil. Surface soils are known to contain indigenous azospirilla in temperate zones [20]. Although samples were taken in this study only during periods of relatively calm winds, high speed winds and storms are frequent in central Ohio and can serve as natural vehicles for transferring azospirilla from one site to the other. In this respect, the climatic conditions prevailing in central Ohio are not unique and are typical of large agricultural areas in North America and Europe.

The movement of *Azospirillum* in air currents in a growth chamber and in a greenhouse containing *Azospirillum*-inoculated plants revealed the possibility that contamination of control plants (a frequent occurrence in inoculation experiments [8]) may also originate from this source. The high *Azospirillum* numbers presented in the growth chamber air (Table 1) are probably of great importance, because the same air has been continuously circulating within the growth chamber; there was no UV irradiation eliminating Gram-negative bacteria, and the soil-surface in pots was usually wet. Thus, random inoculum cells deposited from the air may not instantly disappear, but rather find favorable conditions for multiplication, thereby contaminating plants. Although the numbers of airborne *Azospirillum* in the greenhouse are smaller than in the

growth chamber, even this smaller airborne population resulted in severe contamination of noninoculated plants (Table 3). Only an analysis of every single pot for the presence of the inoculated strain will reveal the magnitude of contamination, an analysis which is difficult and cumbersome to perform in largescale greenhouse pot experiments [10].

This type of study can be performed with many soil or rhizosphere bacteria to provide basic bacteriological information on the lower atmosphere. However, the significance of this study is based on the assumption that *Azospirillum*, as well as other Plant-Growth-Promoting Rhizobacteria (PGPR), technologies are being developed for use in future agriculture [8]. By definition, *Azospirillum* is a typical rhizosphere bacterium, harmless to human and animal health. There is no record of any health hazard caused by any member of this genus [8]; however, this study may have a greater impact for the release into the environment of other soil and rhizosphere microorganisms, such as PGPR organisms employed for bio-control of soil-borne pathogens, especially those that are genetically engineered. When new microbial strains are developed for commercial application, the detection of air contamination from inoculated bacteria should be an important consideration.

Two main sampling techniques are commonly used to monitor airborne dispersal of microorganisms: a volumetric method employing various devices which can give the numbers of dispersal units per a known volume of air [1], and gravity techniques, using gravity deposition on various surfaces [25]. Although both methods are quantitative, the gravity deposition technique is assumed to give an underestimation of the air population [17]. Thus, the actual numbers of *Azospirillum*, and other diazotrophs prevailing in the air of central Ohio, are probably higher than the values monitored in this study. The inability to trap azospirilla and large numbers of other diazotrophs on major highways crossing farmlands needs further study.

In conclusion, the significance of this study is in revealing the possibility that sporeless rhizosphere bacteria of the genus *Azospirillum*, which may have a potential use in agriculture, may have an airborne phase in temperate zones. They can contaminate noninoculated plants in controlled environment experiments via air transfer. In addition, this study proposes that the airborne phase of inoculated bacteria should be considered when man-manipulated soil bacteria are evaluated for release into the environment.

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