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Yield increases in spring wheat (*Triticum aestivum* L.) inoculated with *Azospirillum lipoferum* under greenhouse and field conditions of a temperate region

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Summary Spring wheat (*Triticum aestivum* var. Arkas) was associated with *Azospirillum lipoferum* under greenhouse and field conditions of a temperate region. Controls were treated with autoclaved bacteria. The soils used were: sand, sandy loam, and a peat-clay mixture. In experiments run over a period of three years, there were increases in grain yield, N-yield of the grains, and 1000 grain weight.

Depending from environmental conditions, increase changed from year to year, and within one given year. There was, however, no experiment without positive response to the inoculation. Highest grain yield increase (70%) was found on sand supplemented with P and K only, but up to 32% were also obtained on peat-clay soil containing 0.28% total N. Under greenhouse conditions, one third of technical N-fertilizer could be saved by bacterial activities. With high probability the effects observed have been at least partly due to bacterial N_2-fixation, because the N-yield of the grains was increased (up to 33%), and the most pronounced response was found on sand without any N-fertilizer added.

Introduction

Following the discovery of natural associations of grasses and diazotrophic bacteria, especially Azospirilla, the beneficial role of inoculations with Azospirillum on growth and development of several grasses was studied. A recent review[3] was written by Döbereiner, pioneer on this field. Both negative and positive[1,2,5,8,15,16,17,18,21,22,24,25,28,29,31,33,34,36,37,39] results were obtained. Some of these reports, however, are less convincing because no statistical evaluation was given, and/or the controls were left untreated (*cf.* below). The necessity of appropriate controls will be discussed later.

In wheat, one of our most important nutritive plants, one will be interested mainly in grain yield and grain composition. There are, however, only few publications dealing with these topics. In Brazil, in inoculation studies with *Azospirillum brasilense* there was no significant difference in grain yield between experiment and the untreated control[5]. In Israel, Triticum cultivars responded to inoculation with

Azospirillum brasilense under field conditions with a somewhat increased grain yield[18,25]. In one of these papers[25] the controls were left untreated and no statistical evaluations of the wheat experiment were given, whereas in the other[18] convincing statistics and correct controls were performed. Following inoculation with *Azospirillum lipoferum* under field conditions in a temperate region the grain yield of spring wheat and much more of winter wheat increased significantly. N-yield of the grains was not increased. The controls were untreated, too[31,32].

In vitro studies in our laboratory had revealed a close interaction of spring wheat cultivars and *Azospirillum lipoferum*. Wheat root exudates attracted Azospirilla. Glucose and fructose proved to be chemotactically active substances in the exudates[9]. Wheat plants induced nitrogenase activity in Azospirillum. This induction was possible on media fully supplemented with organic compounds and even with such high levels of N-substances, that nitrogenase activity in bacteria without plant partner was repressed[11]. Using concentration ranges of plant conditioned media it could be shown, that this induction was not due to the removal of repressing N-substances from the medium by the growing plant, but to hitherto unknown factors excreted by the wheat roots. Induction involved the activation of the bacterial nif-operon[12].

Based on these data we tried to investigate, whether there would be a significant increase in grain yield and an influence on grain composition, especially N-yield, of wheat by Azospirillum. The present paper deals with the response of spring wheat to inoculation with *Azospirillum lipoferum* under greenhouse and field conditions in a temperate region.

Materials and methods

Azospirillum lipoferum sp 108 st was obtained from Dr. Döbereiner, EMBRAPA, Rio de Janeiro, Brazil. Stock cultures were kept on NFb agar (15 g agar per l) and subcultured weekly. They were routinely assayed for contamination by plating on NFb medium[4] supplemented with 1 g KNO_3 and 1 mg methionine per l. On these plates the bacteria showed a characteristic pink colour[14].

Subcultures for inoculation were grown in semisolid NFb agar medium (1.75 g agar per l). Inocula were taken from the logarithmic phase of growth: titre 10^8 cells per ml.

Triticum sativum var. Arkas was obtained from Dr. W. Schmütz, Landesanstalt für Saatzucht, University of Hohenheim. 25 grains were sown into one KickBrauckmann pot[19], containing 8 l soil. 20 plants per pot were grown up.

Soil: Three types of soil were used:

1. Washed river sand (Rhine), pH 5.5.

2. Peat-clay, a mixture of 70% white peat and 30% clay routinely used by gardeners under the trade name of "Frustorfer Einheitserde P", containing 6.5 mg P, 22.4 mg K, per 100 g each; total N 0.25–0.28%; organic matter 16%; pH 6.4.

3. Sand-loam, a 1:1 mixture of sand and loam, containing 1.5 mg P, and 6.3 mg K, per 100 g each; total N 0.1%; organic matter 3.5%; pH 6.5

Greenhouse experiments were run in Kick-Brauckmann pots containing 8 l soil. To reduce cross-contamination of the controls, the pots were placed with intervals of 40 cm from each other. Sand and peat-clay mixture were treated with steam of 95°C for one hour, sandy loam was used without being steamed. The greenhouses were heated if the ambient temperature sank below 15°C.

The field experiments were carried out in separated plots, each plot consisting of 10 m^2. Original soil was removed to a depth of 50 cm, the pit was coated with a 0,2 mm plastic foil, and filled with sand and peat-clay mixture, respectively. The foil prevented infiltration from the surrounding soil. In all experiments the final stand was 140 plants per m^2. Four randomized samples of 1 m^2 were harvested from each plot. Total precipitation was 1045 mm in 1982 and 961 mm in 1983.

Inoculations were performed twice, 4 and 5 weeks after sowing. At the time of the first inoculation, wheat plants were in the two leaf stage[6]. 250 ml inoculum per Kick-Brauckmann pot and 0,7 l inoculum per m^2 were used at a time containing 10^8 bacteria per ml. In field experiments 0,7 l inoculum were mixed with 2 l tap water and used for watering on 1 m^3.

Fertilizers were added before sowing, N in form of NH_4NO_3, K in form of KCl, and P in form of Na_2HPO_4. The peat-clay mixture was used with added fertilizers as delivered. Sandy loam was supplied with 2.0 g K and 1.0 g P per Kick-Brauckmann pot (vol. 8 l). Sand was supplied with 1.6 g K, 1.1 g P, and in some experiments with 1 g N per Kick-Brauckmann pot. In field experiments using sand as substrate, 12.4 g K, 8.9 g P and partly additional 6.0 g N, per m^2 each, were given. These fertilizer levels are recommended to farmers in our region[22].

Total N was measured by the Kjeldahl method. Up to 20 Kjeldahls per assay were run.

Statistics: Standard deviations or Students t-test or both were used.

Results

Associations in Kick-Brauckmann jars under greenhouse conditions

During the years 1981–1983, association experiments on different soils, *e.g.* sand, peat-clay mixture, and sand-loam mixture, were carried out under greenhouse conditions in Kick-Brauckmann jars. Experimental plants were inoculated with Azospirilla, controls were treated with autoclaved bacteria. There were no differences between controls treated with autoclaved media with and without bacteria (Table 1).

In associations on sand, extreme differences in vegetative growth between experiment and control could be observed (Fig. 1). Unfortunately, due to an accidental sparrow invasion, it was not possible to determine grain yields. Corresponding associations on sand, however, were run under field conditions, too (*cf.* next section).

In 1981 and 1982, two experiments per summer with a temporal shift of 2 weeks were run on steamed peat-clay mixture. In vegetative growth and development, there were nearly no visible differences between experiments and controls. In only one experiment spike number per plant was a little higher than in control: 2%. In all the assays, however, grain yield, N-content of the grains, and 1000 grain weight were increased compared with the respective controls. As

Table 1. Effect of living Azospirilla suspension, autoclaved Azospirilla suspension, and of fresh Azospirillum culture medium on grain yield of wheat. Each assay consisted of 20 wheat plants per Kick-Brauckmann pot, grown under greenhouse conditions, and was run in 6 parallels. In 1983, due to extreme environmental conditions, wheat growth and development was very poor. Standard deviations are given

		Grain yield in g per assay		
Year	Soil type	Wheat + living Azospirilla	Wheat + autoclaved Azospirilla	Wheat + Medium
1981	Peat-clay	50.98 ± 0.73	43.40 ± 0.46	43.4 ± 0.64
1982a		70.68 ± 1.17	61.02 ± 3.20	60.50 ± 0.80
1982b		59.49 ± 1.69	45.03 ± 3.79	44.10 ± 0.80
1983	Sandy loam	19.50 ± 0.26	18.58 ± 0.35	18.54 ± 0.25

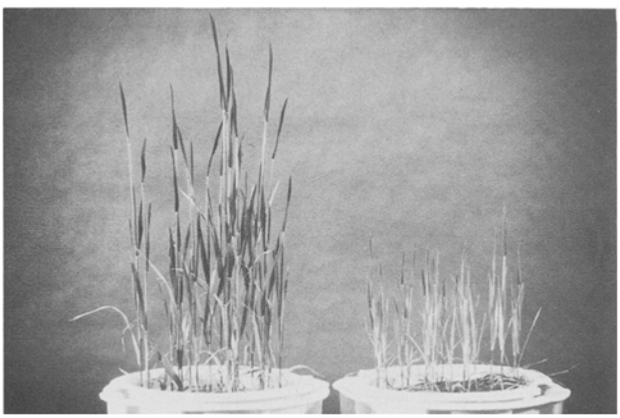

Fig. 1. Growth response of summer wheat to inoculations with *Azospirillum lipoferum*. 20 plants were grown under greenhouse conditions in Kick-Brauckmann jars on sand supplemented with K and P. No N-fertilizer was added. *Left:* experiment (inoculation with Azospirillum). *Right:* control (treatment with autoclaved Azospirilla). The photo was taken 6 weeks after emergence.

expected under non standardized environmental conditions, there were differences from year to year, and within one given year. Increase in grain yield varied from 8 to 32% in N-yield from 10 to 15%, and in 1000 grain weight from 13 to 23% (Fig. 2, Table 2).

These data demonstrate clearly, that inoculations with *Azospirillum lipoferum* benefits yield and N-content of spring wheat grains.

In further experiments the amount of technical N which could be replaced by Azospirillum was investigated. Associations of wheat and *Azospirillum lipoferum* were kept on non steamed sandy loam in Kick-Brauckmann jars. The N content of the soil was 0,1%. Additionally, a concentration range of N-fertilizers was assayed. Controls were treated with autoclaved Azospirilla. Development of the wheat plants

Table 2. Total N-yield of grain and 1000 grain weight in inoculations of wheat with *Azospirillum lipoferum* under greenhouse conditions. Details as for Fig. 2; assay numbers refer to Fig. 2. Standard deviations are given. Additionally, in total N-yield, Students t-test was used. In both assays the differences in total N-yield between experiment and control were statistically significant at $P = 0.01$

	Total N-yield of grain			1000 grain weight	
Assay Nr.	% in grain	mg N per g grain	% increase	g	% increase
3					
C	1.5	15.56 ± 2.10		34.5 ± 4.80	
E	1.7	17.16 ± 0.50	10	38.9 ± 2.70	13
4					
C	1.1	11.60 ± 2.80		26.2 ± 1.60	
E	1.3	13.30 ± 0.57	15	32.2 ± 0.66	23

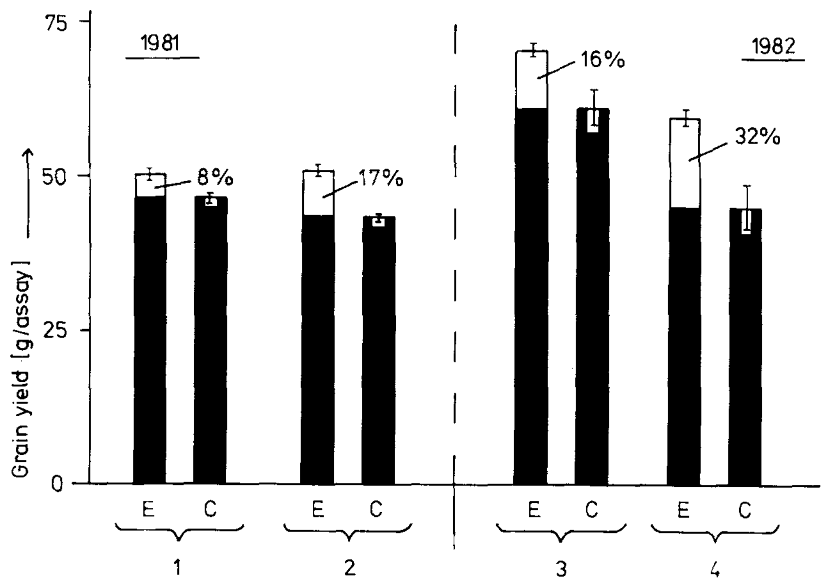

Fig. 2. Grain yield increase in summer wheat following inoculation with *Azospirillum lipoferum*. Each assay consisted of 20 plants grown under greenhouse conditions in a Kick-Brauckmann jar on peat-clay mixture, and was run in 6 parallels. In 1981 and 1982, two independent experimental series were run. The series numbers 3 + 4 refer to the same numbers in Table 2. E = experiment (inoculation with Azospirillum), C = control (treatment with autoclaved Azospirilla). Standard deviations and % increase are indicated.

was bad, probably due to the extreme hot and dry summer of 1983. Consequently, the grain yield was much lower than usual. Despite this, in both experiments and controls grain yield reached a maximum with increasing concentrations of N-fertilizers added. The highest

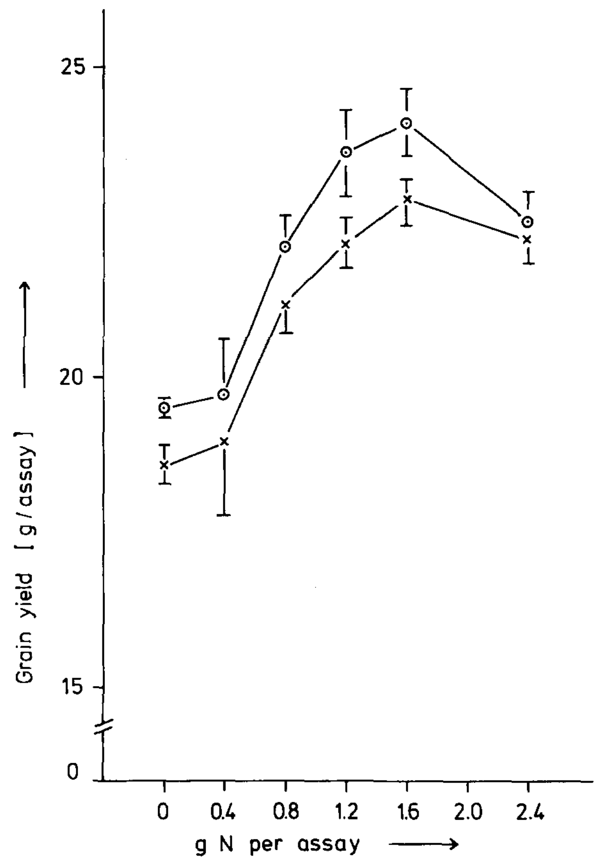

Fig. 3. Grain yield in summer wheat following inoculation with *Azospirillum lipoferum* in relation to N-fertilizers added. Each assay consisted of 20 plants grown in one Kick-Brauckmann pot on sandy loam under greenhouse conditions, and was run in 6 parallels. *Upper curve:* experiment (inoculation with Azospirillum), *lower curve:* control (treatment with autoclaved Azospirilla). Standard deviations are indicated.

level of N-fertilizer tested, 2.4 g, was superoptimal. Grain yield in the experiment was, in most cases significantly, higher than in the controls (Fig. 3). The same applies to 1000 grain weight: in experiment as well as in controls there was a maximum at 1.6 g N per assay, where the experiment showed 11% increase compared with the control. N-yield of the grain, however, showed in experiment and control a linear increase from 0.0 to 2.4 g N added. Maximal increase in the experiment was found at 2.4 g N: 18% compared with the control.

These data allow to calculate the amount of technical N, which could be saved by living Azospirilla. Fig. 4 gives an example, which was taken from the N concentration range showing linear increase

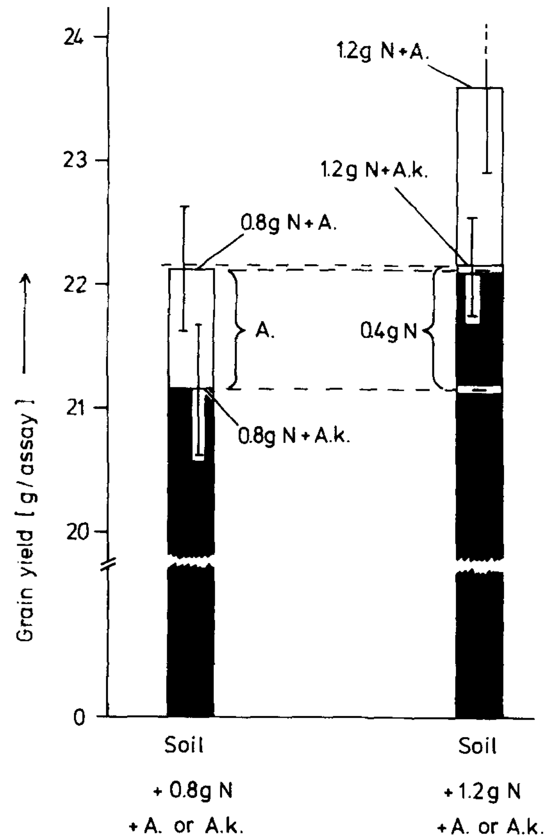

Fig. 4. Replacement of N-fertilizer by *Azospirillum lipoferum* in grain yield increase. Details as for Fig. 3. A = living Azospirilla, A.k. = killed Azospirilla. The same grain yield of a little more than 22 g per assay was obtained by supplying 1.2 g N (plus autoclaved Azospirilla) or by 0.8 g N and living Azospirilla. Therefore, under the conditions used 0.4 g N, *i.e.* one third of the N-fertilizer could be saved.

in grain yield. On the basis of 0.8 g N, the same grain yield could be obtained by either inoculation with Azospirilla or by the supply of additional 0.4 g N. That means from 1.2 g N needed to obtain the grain yield indicated, 0.4 g or one third could be replaced by Azospirlla. This lead to the calculation that at least under the condition mentioned ca. one third of technical N could be saved.

Associations under field conditions

The soils used under field conditions represented two extremes: sand, which was supplemented by phosphate, sodium, and in one experiment by nitrogen, and peat-clay mixture with 0.28% N.

On sand, supplemented only with phosphate and sodium, the

Table 3. Total N-yield of grain and 1000 grain weight in inoculations of wheat with *Azospirillum lipoferum* under field conditions. Details as for Fig. 5 and Table 2; assay numbers refer to Fig. 5. Students t-test (in total N-yield): a = difference statistically significant at $P = 0.01$; b = difference statistically significant at $P = 0.05$

	Total N-yield of grain			1000 grain weight	
Assay Nr	% in grain	mg N per g grain	% increase	g	% increase
1					
C	1.2	12.1 ± 0.20		31.5 ± 0	
E	1.2	12.4 ± 0.10 a	2	32.75 ± 0	4
2					
C	1.2	12.5 ± 0.30		35.2 ± 0.05	
E	1.4	14.2 ± 0.02 a	13	36.7 ± 0	4
3					
C	2.0	20.23 ± 2.30		35.0 ± 0.3	
E	2.6	26.9 ± 3.30 b	33	39.2 ± 0.1	12
4					
C	2.2	22.1 ± 0.04		38.08 ± 0.14	
E	2.2	22.6 ± 0.14 a	2	39.71 ± 0.57	4

differences between experiment and control were spectacular. Vegetative growth in experiment and control differed just as demonstrated in Fig. 1 for jar associations. In grain yield there was an increase of 70% compared with the control. Of course, on sand the absolute values were low. On sand, which was additionally supplied with 6.0 g N/m^2, the grain yield increase was 37%, and on peat-clay mixture with its high N-content about 20% (Fig. 5). Thus in 1983 a grain yield of 3.85 tons per ha was obtained on peat-clay mixture in the experiment, as compared to 3.17 tons per ha in the control. The value of 3.85 tons per ha coincided with those obtained by farmers in our region on fully fertilized soil.

Increase in N-content and 1000 grain weight were obtained as well. As shown in Table 3, they were less pronounced than the increase in grain yield.

These values demonstrate that even in temperate regions an inoculation with *Azospirillum lipoferum* leads to an increase in grain yield and total N of spring wheat.

Discussion

As mentioned earlier, in some inoculation experiments under greenhouse or field conditions the controls were left untreated. Using high titre inocula, a considerable amount of bacterial N could be added

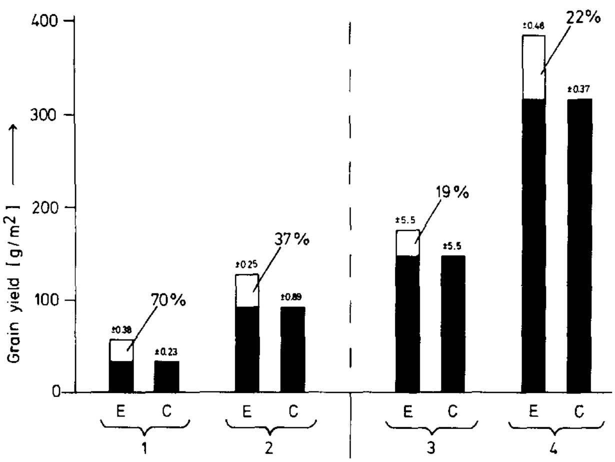

Fig. 5. Grain yield increase in summer wheat following inoculation with *Azospirillum lipoferum*. Wheat plants were grown under field conditions in 10 m^2 plots on sand and peat-clay mixture, respectively. The series numbers refer to the same numbers in Table 3. 1 = sand supplemented with P and K, 2 = sand supplemented with P, K, and N, 3 and 4 = two parallel experimental series on peat-clay mixture. E = experiment (inoculation with Azospirillum), C = control (treatment with autoclaved Azospirilla). Standard deviations are given on top of the columns. % increase are indicated.

to the soil. Furthermore, if bacterial suspensions as usual are sprayed or poured on the field or pot, one has to take into consideration the possible beneficial role of substances in the nutrient broth. These could be components of the medium itself or factors excreted by bacteria. For instance, Azospirilla are able to convert tryptophan into indole-3-acetic acid[30]. Therefore, it seems to be a mistake to use untreated controls.

One possible control could be the use of autoclaved bacterial suspensions. However, such a control could not be considered a fully satisfactory one. Firstly, one has to exclude that physiological active substances were destroyed by autoclaving. Secondly, it will be difficult to differentiate between a bacterial activity due to the excretion of growth promoting substances, and a benefit due to bacterial N_2-fixation.

The ideal control would be to use nif^--mutants of the respective bacteria, which are normal as for their production of plant growth factors. There are claims to have obtained nif^--mutants of *Azospirillum brasilense*[20]. More recent investigations, however, revealed an extreme rarity of nif^--mutants in mutation experiments using *A. brasilense*[13]. With *Azospirillum lipoferum*, the species used in our experiments, it was not yet possible to get nif^--mutants[14]. Furthermore, it should be proven that nif^--mutants, which should be used as controls in inoculation studies, showed no disturbance in the production of plant growth factors. Such mutants are not yet available even from *A. brasilense.*

Therefore, the treatment with autoclaved bacterial suspensions seemed to be the best practicable control at least at the moment. It should be added, that most of the auxins including indole-3-acetic acid which could be produced by Azospirilla, are rather thermostable if they are not autoclaved in strong alkaline or acid media[27]. In our investigations, there were no differences between autoclaved media with and without Azospirilla (Table 1). With these two different controls it could be excluded that the response of wheat plants to *Azospirillum lipoferum* was due to the bacterial culture medium, to bacterial N, to substances excreted by Azospirilla until inoculation, or to the growth of other microorganisms using dead Azospirilla as a feeder-layer and possibly inhibiting the plant growth. Therefore, any effect observed must be due to the activity of living bacteria following inoculation.

As mentioned in the introduction, there are several publications dealing with beneficial effects of inoculations with Azospirillum on growth and development of wheat. Using appropriate controls, these data were confirmed by our results: grain yield, total N of the grain, and 1000 grain weight were significantly increased in the greenhouse as well as under field conditions of a temperate region. As just discussed, these effects must be due to the activity of living Azospirilla. There are, however, three possible mechanisms of such an activity: N_2-fixation, production of plant growth substances, and interaction with nitrate assimilation of the plant[26]. Additionally, the possibility of quite indirect bacterial effects on wheat growth and development cannot be completely excluded.

There are some data which could be explained by plant growth substances excreted by Azospirilla[8,21,22,31,37]. For instance, in associations of pasture grasses with *Azospirillum lipoferum* on semisolid agar medium, no increase in growth rate and nitrogen content of the grasses was found, but an increase in the ratio of root to shoot dry weight. This could have been due to an effect of phytohormones produced by the bacteria[22]. In field inoculations of wheat with *A. lipoferum,* significant increases in grain yield were obtained. Total N-yield was not increased. Therefore, and because grain yield increase coincided with a higher tillering ratio (tillers per plant), it had been assumed that the production of growth subtances by the bacteria could be more important than N_2-fixation[31].

There are, however, several arguments for a participation of N_2-

fixation in the effects observed in the wheat – Azospirillum associations described here. First, using the acetylene reduction assay, nitrogenase activity could be detected in *Azospirillum lipoferum* associated with wheat on soil as substrate. However, this point should not be overemphasized. There are difficulties in extrapolating the data from acetylene reduction assays to the actual dinitrogen fixation[35], and before all, N_2-fixation does not necessarily mean that the wheat plants benefit from the nitrogen fixed by the Azospirilla. $^{15}N_2$-studies will be needed to get full evidence.

On the other hand, and more important, total N-yield of the grains was increased in associations with Azospirillum. This effect, however, could be eventually related to an interaction of plant and Azospirillum in the utilization of soil nitrate, as assumed, for instance, for rice which had been inoculated with several Azospirillum strains[38].

Finally, the most spectacular increases in vegetative growth and grain yield, and increases in total N of the grains as well, were obtained on sand with no N-fertilizer added inoculated with *Azospirillum lipoferum*. It will be difficult to explain these results without participation of bacterial N_2-fixation.

Therefore, the effects obtained in these investigations should have been at least due partly to the N_2-fixation of the Azospirilla. This does not exclude an additional bacterial benefit by producing plant growth factors[8,22,31,37], or, hitherto less substantiated, in soil nitrate utilization[7,37].

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References

1 Bouton J H, Smith R L, Schank S C, Burton G W, Tyler M E, Little R C, Gallaher R N and Quesenberry K H 1979 Response of pearl millet inbreds and hybrids to inoculation with *Azospirillum brasilense*. Crop Sci. 19, 12–16.

2 Cohen E, Okon Y, Kigel J, Nur I and Henis Y 1980 Increase in dry weight and total nitrogen content in *Zea mays* and *Setaria italica* associated with nitrogen-fixing *Azospirillum* spp. Plant Physiol. 66, 746–749.

3 Döbereiner J 1983 Dinitrogen fixation in rhizosphere and phyllosphere associations. *In* Encyclopedia of Plant Physiol., New Series, vol. 15A. Eds. A Läuchli and R L Bieleski. Springer Verlag, Berlin. pp 330–350.

4 Döbereiner J and Day J M 1976 Associative symbiosis in tropical grasses: characterization of microorganisms and dinitrogen fixation sites. *In* Proc. of the First Intern. Symp. on Nitrogen Fixation. Eds. W E Newton and C J Nyman. Washington State Univ. Press, Pullman. pp 518–538.

5 Döbereiner J and De-Polli H 1980 Diazotrophic rhizocoenoses. *In* Nitrogen Fixation. Eds. W D P Stewart and J R Gallon. Academic Press, London. pp 301–333.

6 Fischbeck G, Heyland K-U and Knauer N 1982 Spezieller Pflanzenbau. Ulmer, Stuttgart. p 394.
7 Freitas de J L M, Pereira P A A and Döbereiner J 1981 Effect of organic matter and *Azospirillum* spp. strains in the metabolism of nitrogen in *Sorghum vulgare*. *In* Associative N_2-Fixation, vol. 1. Eds. P B Vose and A P Ruschel. CRC Press, Boca Raton. pp 155–163.
8 Glatzle A and Martin P 1981 Some interactions between *Azospirillum* spp. and grass seedlings. *In* Associative N_2-Fixation, vol. 1. Eds. P B Vose and A P Ruschel. CRC Press, Boca Raton. pp 27–36.
9 Heinrich D and Hess D 1984 Chemotactic attraction of *Azospirillum lipoferum* by wheat roots: activity of wheat conditioned media, and characterization of some attractants. Can. J. Microbiol. submitted for publication.
10 Hess D 1982 Induction of nitrogenase activity in Azospirillum by wheat. *In* Azospirillum Genetics, Physiology, Ecology. Ed. W Klingmüller. Birkhäuser, Basel. pp 69–74.
11 Hess D and Kiefer S 1981 Induction of bacterial nitrogenase activity in *in vitro* associations: a comparison of the inducing capabilities of *Triticum aestivum* and *Sorghum nigricans*. Z. Pflanzenphysiol. 101, 15–24.
12 Hess D, Kemmner J, Heinrich D, Jekel S and Pudil H 1984 Nitrogenase induction in *Azospirillum lipoferum* by wheat plants: transfilter induction, induction by wheat conditioned media, nif gene activation, and characterization of some inducing factors. Manuscript in preparation.
13 Jara P, Quiviger B, Laurent P and Elmerich C 1983 Isolation and genetic analysis of *Azospirillum brasilense* nif^--mutants. Can. J. Microbiol. 29, 968–972.
14 Jörger R 1983 Untersuchungen zur Gewinnung von nif^--Mutanten von Azospirillum. University Hohenheim (diploma work), Stuttgart, 114 p.
15 Kapulnik Y, Kigel J, Okon Y, Nur I and Henis Y 1981a Effect of Azospirillum inoculation on some growth parameters and N-content of wheat, Sorghum and Panicum. Plant and Soil 61, 65–70.
16 Kapulnik Y, Sarig S, Nur I, Okon Y, Kigel J and Henis Y 1981b Yield increases in summer cereal crops in Israeli fields inoculated with Azospirillum. Expl. Agric. 17, 179–187.
17 Kapulnik Y, Okon Y, Kigel J, Nur I and Henis Y 1981c Effects of temperature, nitrogen fertilization, and plant age on nitrogen fixation by *Setaria italica* inoculated with *Azospirillum brasilense* (strain cd). Plant Physiol. 68, 340–343.
18 Kapulnik Y, Sarig S, Nur I and Okon Y 1983 Effect of Azospirillum inoculation on yield of field-grown wheat. Can. J. Microbiol. 29, 895–899.
19 Kick K and Große-Brauckmann E 1961 Über die Konstruktion eines Vegetationsgefäßes aus Plastik. Z. f. Pflanzenern. Düngung Bodenk. 95, 52–55.
20 Lemos M V F, Santos D S Trabulsi L R and Azevado J L 1981 Possible role of plasmid deoxyribonucleic acid in nitrogen fixation in *Azospirillum brasilense*. *In* Associative N_2-Fixation, vol. 1. Eds. P B Vose and A P Ruschel. CRC Press, Boca Raton. pp 63–68.
21 Lethbridge G and Davidson M S 1983 Root-associated nitrogen-fixing bacteria and their role in the nitrogen nutrition of wheat estimated by ^{15}N isotope dilution. Soil Biol. Biochem. 15, 365–374.
22 Martin P and Glatzle A 1982 Mutual influence of *Azospirillum* spp. and grass seedlings. *In* Azospirillum Genetics, Physiology, Ecology. Ed. W Klingmüller. Birkhäuser, Basel. pp 108–120.
23 Nieder H (Ed.) 1978 Düngungsratschläge für den Bauernhof. Fachverband Stickstoffindustrie, Düsseldorf, 136 p.
24 Nur I, Okon Y and Henis Y 1980 An increase in nitrogen content of *Setaria italica* and *Z. Mays* inoculated with Azospirillum. Can. J. Microbiol. 26, 482–485.
25 Okon Y 1982 Field inoculation of grasses with Azospirillum. *In* Biological Nitrogen Fixation Technology for Tropical Agriculture. Eds. P H Graham and S C Harris. CIAT, Cali. pp 459–467.
26 Patriquin D G, Döbereiner J and Jain D K 1983 Sites and processes of association between diazotrophs and grasses. Can. J. Microbiol. 29, 900–915.

27 Posthumus A C 1971 Auxins. *In* Effects of Sterilization on Components in Nutrient Media. Eds. J van Bragt, D A A Mossel, R L M Pierik and H Veldstra. Agricultural University, Wageningen. pp 125–128.
28 Rennie R J and Larson R I 1979 Dinitrogen fixation associated with disomic chromosome substitution lines ofspring wheat. Can. J. Bot. 57, 2771–2775.
29 Rennie R J and Larson R I 1981 Dinitrogen fixation associated with disomic chromosome substitution line of spring wheat in the phytotron and in the field. *In* Associative N_2-Fixation, vol. 1 Eds. P B Vose and A P Ruschel. CRC Press, Boca Raton. pp 145–154.
30 Reynders L and Vlassak K 1979 Conversion of tryptophan to indoleacetic acid by *Azospirillum brasilense.* Soil Biol. Biochem. 11, 547–548.
31 Reynders L and Vlassak K 1982 Physio-ecological aspects and agricultural importance of Azospirillum – plantroot associations. *In* Azospirillum Genetics, Physiology, Ecology. Ed. W Klingmüller. Birkhäuser, Basel. pp 121–129.
32 Reynders L and Vlassak K 1982 Use of *Azospirillum brasilense* as biofertilizer in intensive wheat cropping. Plant and Soil 66, 217–223.
33 Smith R L, Bouton J H, Schank S C, Quesenberry K H, Tyler M E, Milam J R, Gaskins M H and Littell R C 1976 Nitrogen fixation in grasses inoculated with *Spirillum lipoferum.* Science 193, 1003–1005.
34 Smith R L, Schank S C, Bouton J H and Quesenberry K H 1978 Yield increases of tropical grasses after inoculation with *Spirillum lipoferum.* Ecol. Bull. 26, 380–385.
35 Stewart W D P, Rowell P and Lockhart C M 1979 Associations of nitrogen-fixing prokaryotes with higher and lower plants. *In* Nitrogen Assimilation of Plants. Eds. E J Hewitt and C V Cutting. Academic Press, London. pp 45–66.
36 Subba Rao N S 1981 Response of crops of Azospirillum inoculation in India. *In* Associative N_2-Fixation, vol. 1. Eds. P B Vose and A P Ruschel. CRC Press, Boc Raton. pp 137–144.
37 Tien T M, Gaskins M H and Hubbel D H 1979 Plant growth subtances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L). Appl. Environ. Microbiol. 33, 1016–1024.
38 Villas Boas F C S and Döbereiner J 1981 Nitrogenase and nitrate reductase activities in rice plants inoculated with various Azospirillum strains. *In* Associative N_2-Fixation, vol. 2. Eds. P B Vose and A P Ruschel. CRC Press, Boca Raton. pp 231–239.
39 Vlassak K and Reynders L 1981 Agronomic aspects of biological dinitrogen fixation by *Azospirillum* spp. in temperate region. *In* Associative N_2-Fixation, vol. 1. Eds. P B Vose and A P Ruschel. CRC Press, Boca Raton. pp 93–101.