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Plant growth stimulation by inoculation with symbiotic and associative rhizosphere microorganisms

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Abstract. Selected *Rhizobium* bacteria, arbuscular mycorrhiza-forming (AM) fungi and associative bacteria have been shown to stimulate the growth of legumes, gramineae and cruciferae in field experiments on different soil types in temperate regions. A combination of microorganisms with different metabolic capacities (N_2-fixation, P-mobilization; production of phytohormones and antibiotics) can partly surpass the effect of single inoculations, or can produce a positive effect where single inoculations are ineffective. Growth stimulation by inoculation requires microorganisms with phytoeffective metabolic characteristics and the ability to survive in the rhizosphere during the growth period. Another prerequisite is an adequate supply of plant assimilates for the production of microbial phytoeffective metabolites. Type of inoculum, method of inoculation and agricultural measures can influence the effect of the inoculation. Research is necessary to extend our knowledge both of basic principles, and about using microorganisms in practice.
Key words. Rhizosphere microorganisms; plant growth promotion; N_2-fixation; P-mobilization; phytohormones; antagonism; root colonization; growth conditions.

Importance of rhizosphere microogranisms for plant growth

Rhizosphere microorganisms can stimulate the growth of plants by mobilization of nutrients and production of phytoeffective metabolites, protecting the plants from pathogens, decomposing toxic substances and increasing the stress tolerance, as well as by forming and stabilizing soil structures. They can also inhibit the plant's growth by parasitism, or by release of phytotoxins.

It is the aim of international research to make better use of natural microbial resources in a sustainable form of agriculture, in order to reduce the input of fertilizers and pesticides and thus the pollution of soils and ground water, as well as to decrease the energetic costs and the environmental pollution connected with the production of agrochemicals.

The heterotrophy of rhizosphere organisms demands organic compounds to supply energy for the production of phytoeffective metabolites. In the rhizosphere, these are primarily exudates, lysates and mucilages from the roots, which can differ according to the plant species. In addition, root residues and organic fertilizers can stimulate the microbial activity in the soil. Since young plant roots are preferentially colonized by bacteria surviving on organic soil substances, similar groups of microorganisms are found in these two environments[35].

A selective influence on the rhizosphere microflora can be exercised by the rotation of crops, but not by organic fertilization and tillage[37]. The cultivation of legumes enhances the propagation of N_2-fixing *Rhizobium* bacteria (e.g. ref. 53). The concentrated cultivation of individual crop species can, however, also induce the propagation of root pests, resulting in yield reductions[65].

The influence on crops of the wild microbial population occurring in the soil can, for the most part, not be controlled[18], because the soil contains a broad spectrum of microorganisms, ranging from deleterious to growth-promoting ones, which all use the plant roots and their organic deposits as a source for their own development. This microorganism community is more or less in balance. However at an early stage of root development it does seem possible to manipulate this balance, in order to promote plant growth by inoculation with selected rhizosphere microorganisms. Attempts are therefore made to stimulate plant growth by inoculation with efficient rhizosphere microorganisms.

The following questions need to be answered:

- Which characteristics of microbial metabolism and colonization are of special importance for plant growth stimulation?
- Are growth-stimulating effects specific for crop species, microorganism species and soil types?
- Can the effect of combined inoculations with different microorganisms surpass that of individual inoculations?
- Which prerequisites must be fulfilled under field conditions to obtain a positive inoculation effect?

This publication will investigate possibilities of and prerequisites for using rhizosphere microorganisms for promoting the growth of agronomic cultures under field conditions in temperate climates, on the basis of international results as well as experiments of the authors' group with selected *Rhizobium* species, AM fungi and associative bacteria on legumes, gramineae and cruciferae.

Materials and methods

Investigations were carried out by the authors' group with pea, alfalfa, lupin, broad bean and maize from 1981 to 1993 in pot and field experiments on different soils (loamy sand, sandy loam, chernozem). Inoculation experiments were made with selected plant-specific *Rhizobium* strains, E163 with pea, mel8 with alfalfa, lup84 with lupin, and A150 with broad bean. *Pseudomonas fluorescens* strain PsIA12, *R. leguminosarum* bv. *trifolii* strain R39, and fast-infecting arbuscular mycorrhiza-forming (AM) fungi (VAM3, VAM5) were inoculated alone and in combination with the symbiotic *Rhizobium* strains.

(For detailed description of strain selection and inoculation experiments see refs 31–33, 36, 38, 39 and 72.)

All results were tested by ANOVA for completely randomized blocks and the LSD test for statistical significance ($\alpha = 0.05$). For comparison of a series of experiments the relative values were tested by means of their confidence intervals for significance ($\alpha = 0.05$) against the non-inoculated control.

Importance of microbial metabolic performance, in dependence on plant colonization, for plant growth

Important metabolic features of microbes are:

- nutrient mobilization by fixation of atmospheric nitrogen or mobilization of soil phosphates;
- stimulation of the nutrient uptake of roots by microbial phytohormones and vitamins;
- protection against soil-borne plant pathogens and deleterious bacteria.

For an effective growth stimulation, a close interaction between efficient microorganisms and host plants is necessary as a prerequisite for the utilization by the partners of plant assimilates or microbial metabolites respectively.

Biological fixation of atmospheric nitrogen

The production of nitrogen fertilizers demands a high energy input. For the production of 1 kg of fertilizer nitrogen, approximately 75 MJ are necessary[60]. The application to fields carries with it the risk that a large part of the mineral nitrogen will be leached out as nitrate, or escape into the atmosphere after denitrification to N_2 or nitrous oxides.

Only microorganisms possessing the enzyme nitrogenase are able to utilize atmospheric nitrogen. The energy necessary for N_2-fixation is supplied by the photosynthesis of the host plants. *Rhizobium* bacteria can cover up to 90% of the nitrogen requirements of legumes from the atmosphere[20, 52]. Inoculation with selected *Rhizobium* bacteria has repeatedly been shown to enhance the growth of legumes in different countries by 5–20%[1, 53, 67]. There are, however, also reports of studies in which no effect of an inoculation was found[19].

In our experiments, three of 514 *R. meliloti* isolates (<1%) repeatedly increased the dry matter yield of alfalfa in field experiments performed during 2 years. The effectivity of the inoculation was higher on nutrient-poor loamy sand (1–2 tons of dry matter yield per hectare) than on sandy loam and chernozem (0.5–0.9 tons of dry matter yield per hectare, table 3)[41].

In pea, 3 of 171 (2%) of *R. leguminosarum* isolates repeatedly produced growth stimulation and increases in grain yield[38]. In field experiments, an average grain yield increase of 0.2 tons per hectare was achieved. Due to root damage caused by fungal pests, the effect of an inoculation was lower on loamy sand in this case, and higher on chernozem, where the plants were less infected by pests.

The yield increase caused by the inoculation with *Rhizobium* spp. was at least equivalent to a nitrogen fertilization of 40 kg N per hectare. An additional nitrogen fertilization did not improve the effect of an inoculation with efficient *Rhizobium bacteria*[38]. The nitrogen fixed by the *Rhizobium*-legume symbiosis can be utilized by non-leguminous plants e.g. in legume-grass mixtures[40, 63], or by the following crop in the case of an optimal crop rotation regime[48, 55].

Non-symbiotic rhizosphere bacteria (e.g. *Azospirillum* spp., *Pseudomonas* spp., *Bacillus* spp.) also contain nitrogenase[11, 23, 44]. No proof could be found so far that growth stimulation in the field by these nitrogenase-active, associative bacteria are definitely caused by their N_2-fixation activity.

Phosphorus mobilization

Besides nitrogen, phosphorus is an important plant nutrient with considerable effects on yield[25]. Only 10–20% of fertilizer phosphorus can be utilized by the plants, while the major part is deposited in the soil as Ca-, Fe-, or Al-phosphates[58].

The phosphorus demand of plants cannot be sufficiently covered by diffusion. Rhizosphere microorganisms (e.g. *Bacillus* spp., *Penicillium* spp.) can, however, solubilize inorganic phosphorus compounds by excreting organic acids, and mineralize organic phosphorus by phosphatases, thus making them utilizable for plants[17, 30, 49]. So far, it has not been possible to prove that growth stimulations under field conditions were exclusively caused by P-mobilization of bacteria.

AM fungi are thought to be more effective in the mobilization of P-reserves in the soil[22, 27]. With their mycelium, they enlarge the exploitation area of the roots, and they can increase the phosphatase activity per unit root length. AM fungi can enhance the P-uptake of chickpea[69,70] and clover[57]. Inoculations with AM fungi increased the yield of cereals[46], maize[3,22], and legumes[28,38,39]. Quickly-infecting isolates had a competitive advantage[33].

Protection from phytopathogenic microorganisms

Crop growth may be depressed by soil-borne plant pathogenic fungi and other growth-inhibiting microorganisms (e.g. toxin producing *Pseudomonas* spp.). They can be enriched by a concentrated cultivation of particular species in the crop rotation[2,65,66]. Protection against these pests can partly be achieved by antagonistic microorganisms. This antagonism is considered to be due to competition for essential nutrients (P, Fe), production of antibiotics, or parasitism of the pathogens. Another possibility is to increase the resistance or tolerance of plants by microbial stimulation of seedling development, or by microbial metabolites[43,71]. Protective effects were achieved with *Trichoderma* spp., *Pseudomonas* spp. or *Bacillus* spp.[6,10], and AM fungi[4,26,43] and *Rhizobium* bacteria[13,39] can also protect crops from damage caused by root pests. Possibilities of biological control of soil-borne plant pathogens have been summarized[43].

Stimulation of nutrient uptake by phytohormones

Phytohormones (auxin, cytokinin, gibberellin) can stimulate root development[12]. They are produced not only by plants but also by rhizosphere bacteria in vitro, e.g. *Azotobacter* spp., *Agrobacterium* spp., *Streptomyces* spp.[45], and by AM fungi[8]. The question of whether bacterial phytohormones are able to stimulate root growth in situ is still under discussion. However, there are many references to effects of phytohormone-producing microogranisms on nutrient uptake by influence on the root surface and on the nutrient acquisition per unit root surface, e.g. by an increased phosphatase activity[16,59]. These effects can, however, not always be attributed directly to the microbial phytohormones, because a variety of interactions exists in the nonsterile rhizosphere. Like auxins, nitrites from denitrification can also promote the root development[73].

Multiple effects of microbial inoculation

Since some of the diazotrophic (e.g. *Rhizobium* spp., *Azospirillum* spp.) and phosphate-mobilizing microorganisms (AM fungi, *Pseudomonas* spp.) also produce phytohormones, reduce nitrate or have antagonistic effects, an unambiguous determination of the factors causing growth stimulation is not always possible. Growth stimulation by *Rhizobium* spp. (see above 'Biological fixation of atmospheric nitrogen'), AM fungi (see above 'Phosphorus mobilization') and nonsymbiotic microorganisms (table 1) can be caused by different characteristics of the microbial metabolism[32,38]. A strict specialization of the microorganisms for particular host plants cannot always be established.

The inoculated microorganisms can, furthermore, have an effect on the rhizosphere wild population and the overall metabolism of the plants. A *Rhizobium* inoculation, for instance, did not only stimulate the nitrogen uptake of alfalfa, but also the P, K, and Mg uptake in field experiments (table 3). In another case, AM fungi enhanced the nodulation of wild *Rhizobium* populations and the nitrogen uptake of alfalfa without an additional *Rhizobium* inoculation[39].

Colonization characteristics of growth-stimulating microorganisms

Since seedling roots are scarcely colonized with microorganisms in the beginning, they provide a niche for the colonization of desired organisms. The survival and propagation of the inoculated microorganisms in the rhizosphere is a prerequisite of growth-stimulating effects. Close contact to the host plant not only favours the exchange of nutrients and active substances between the partners, but also protects the microogranisms from antagonists.

Survival during the growth period is favoured by

- quick propagation in the rhizosphere,
- mobility along the growing roots,
- colonization of the inner root tissues (intercellular spaces).

Along with the research on symbiotic organisms (*Rhizobium* spp., AM fungi), attention is increasingly being drawn to metabolically active, associative rhizosphere bacteria which partly also colonize the endorhizosphere. Among them are *Pseudomonas* spp., *Rhizobium* spp., and *Enterobacter* spp.[5,7,9,21,24,32,47,51,54,72]. Selected strains of these genera repeatedly stimulated the growth of various crops at different yield levels[45].

In our own field experiments, a *Pseudomonas fluorescens* strain (PsIA12) isolated from wheat roots[32], and a strain of *Rhizobium leguminosarum* bv. *trifolii* (R39) isolated from clover[31], stimulated the growth of cereals, maize, rape, oilradish, mustard, *Phacelia*, and pea (table 1). In vitro, boths trains have the potential ability to produce phytohormones (cytokinin, auxin). R39 is able to fix N_2, and PsIA12 to mobilize phosphate, exercise antagonistic effects and reduce nitrate. They are relatively osmotolerant and propagate at pH 5-8 (ref. 39).

The capacity of these microorganisms to survive in the rhizosphere of the different crops is of crucial importance[31,39,72]. The colonization densities can, however, differ according to bacteria and crop species. Electron microscopic investigations (figure) showed that:

- The rhizoplane of lupin, in contrast to that of pea, is densely colonized.
- PsIA12 is able to colonize the root surface and the root tip mucigel as well as intercellular spaces of the

Table 1. Growth promotion of different crops by *Pseudomonas fluorescens* (PsIA12) and *Rhizobium leguminosarum* bv. *trifolii* (R39).

Bacterial strain	Crop	Number of experiments	Shoot dry matter		
			Control $t \cdot ha^{-1}$	Extra yield $t \cdot ha^{-1}$	%
PsIA12	maize	4	15.89	1.01^{+}	6
	rape	2	1.95	0.38	19
	oilradish	2	2.25	0.40^{+}	18
	mustard	2	2.33	0.74^{+}	30
	phacelia	1	3.00	1.12^{+}	37
	pea	1	3.36	0.92^{+}	27
R39	maize	5	16.92	1.14^{+}	7
	spring wheat	5	5.16	0.34^{+}	8
	winter wheat	1	11.98	0.21	2
	spring barley	1	9.58	0.52^{+}	6
	red clover	6	15.97	1.18^{+}	7

Field experiments on loamy sand (1985–1993).
$^{+}$Significant at $p < 0.05$.

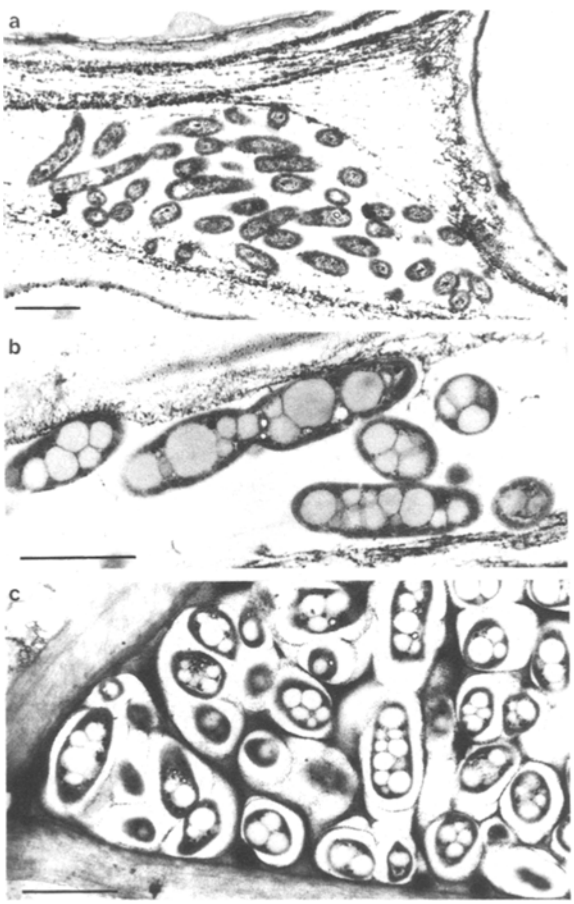

Figure. Colonization of white lupin roots with associative, plant growth-promoting rhizosphere bacteria. Transmission electron micrographs, bar = 1 µm. *a* Dense colonization of the root tip mucigel by *Rhizobium leguminosarum* bv. *trifolii* strain R39 and *b Pseudomonas fluorescens* strain PsIA12. *c* Strain PsIA12 in the intercellular space of the living cortex, zone of branching (micrographs by W. Wiehe).

cortex, and places where the cortex is ruptured as a result of the penetration of emerging lateral roots.

—R39 colonizes the mucigel and the rhizoplane of the lateral root zone and also the cortex ruptures resulting from emerging laterals. In the root hair zone, single bacteria attach to the epidermal cells in a manner characteristic of *Rhizobium* bacteria, but nodulation is not observed.

—Nodulating *Rhizobium* strains (lup84, E163), specific for their host plants, do not colonize the mucigel.

From the interactions between metabolic activity, root colonization and growth stimulation, the hypothesis can be derived that the microbial metabolites are especially effective in root zones of pronounced growth like the mucigel of the root tip, the root hair zone, the intercellular spaces of the cortex and the cortex ruptures resulting from emerging lateral roots. The colonization of actively growing root zones by effective microorganisms is therefore of special importance[5,72]. Selected rifampicin-resistant mutants of the *Pseudomonas* strain PsIA12 and the *Rhizobium* strain R39 survived and became established in the rhizospheres of lupin and pea during the whole vegetative period in a field experiment on loamy sand in the year 1993 (not published). Soil factors such as clay content, pore volume and root protozoans can exercise an influence on the survival of the inoculated organisms[14,29].

Growth promotion by combined inoculation with microorganisms with different metabolic characteristics

Since some growth-promoting microorganisms preferentially produce certain metabolites and colonize different niches on the plant roots, efforts have been made to enlarge their growth-stimulating effect by combining N_2-fixing, host-specific *Rhizobium* bacteria with P-mobilizing AM fungi, or associative phytohormone producing or antagonistic microorganisms.

Combined inoculation with *Rhizobium* bacteria and AM fungi

In our experiments, a combined inoculation with selected *Rhizobium* strains together with AM fungi iso-

Table 2. Growth promotion of alfalfa, pea and maize by inoculation of *Rhizobium* bacteria (Rhiz) and AM fungi (AM).

Crop	Soil type	Number of experiments	Control yield $t \cdot ha^{-1}$	Extra yield with inoculation		
				AM $t \cdot ha^{-1}$	Rhiz $t \cdot ha^{-1}$	AM + Rhiz $t \cdot ha^{-1}$
			Dry matter yield			
Alfalfa	Loamy sand	3	14.86	2.63^{+}	1.92^{+}	2.88^{+}
	Sandy loam	2	16.01	0.06	0.93	1.84^{+}
			Seed yield			
Pea	Loamy sand	3	3.57	0.54^{+}	0.35	0.68^{+}
			Seed yield			
Maize	Loamy sand	6	6.74	1.16^{+}		

Field experiments on loamy sand and sandy loam (1987–1993).
$^{+}$Significant at $p < 0.05$.

Table 3. Influence of *Rhizobium*- and AM-fungi-inoculation on alfalfa dry matter yield and nutrient uptake.

Dry matter & nutrients	Inoculated microorganisms		
	AM	Rhiz	AM + Rhiz
Dry matter	113^{+}	111^{+}	118^{+}
N	113^{+}	115^{+}	122^{+}
P	114^{+}	115^{+}	124^{+}
K	115^{+}	107	113^{+}
Mg	113^{+}	107	116^{+}

Means of 4 field experiments on loamy sand and sandy loam. Control = 100% (1987–1990).
$^{+}$Significant at $p < 0.05$.

lates repeatedly surpassed the effect of *Rhizobium* inoculates in alfalfa and pea (table 2).

Combined inoculation of alfalfa improved the acquisition of nutrients: nitrogen from the atmosphere, and P, K and Mg from the soil (table 3). Similar results with cowpea were reported[68]. Combined inoculations with *Rhizobium* bacteria and AM fungi equally had positive effects on plants used for landscaping spoil-heaps and dunes[26, 56].

Inoculation with *Rhizobium* or AM fungi combined with associative bacteria

In our experiments with peas, reproducible increases in the effectiveness of the host-specific *Rhizobium* bacteria were achieved by combining them with *Rhizobium leguminosarum* bv. *trifolii* strain R39 (table 4). A combination with *Pseudomonas fluorescens* strain PsIA12 was partially successful. These results were obtained not only on nutrient-poor loamy sand but also on nutrient-rich chernozem. Both strains promoted especially the development of seedlings, by enlarging the root surface (length and branching), and effected a better protection from root pests. Since the strain R39 increased the leghaemoglobin concentration in the nodules of plants in the young stage and after flowering, growth-stimulating effects can be attributed to a prolongation of the N_2-fixation phase[36]. The effect of a *Rhizobium* inocula-

Table 4. Growth promotion of pea, alfalfa and broad bean by specific symbiotic *Rhizobium* strains (Rhiz) in combination with associated bacteria (R39, PsIA12).

Crop	Soil type	Number of experiments	Control yield	Extra yield with inoculation		
			$t \cdot ha^{-1}$	Rhiz $t \cdot ha^{-1}$	Rhiz + R39 $t \cdot ha^{-1}$	Rhiz + PsIA12 $t \cdot ha^{-1}$
			Seed Yield			
Pea	Loamy sand	4	2.72	0.22	0.45^{+}	0.37
	Sandy loam	3	4.63	0.10	0.10	0.21^{+}
	Chernozem	3	3.55	0.29	0.52^{+}	0.30
Broad bean	Sandy loam	2	6.26	0.15	0.34^{+}	0.33^{+}
			Dry matter yield			
Alfalfa	Loamy sand	2	18.98	1.68^{+}		2.34^{+}
	Sandy loam	2	16.01	0.93		1.42^{+}

Field experiments on loamy sand, sandy loam and chernozem (1989–1993).
$^{+}$Significant at $p < 0.05$.

tion of broad beans could be equally improved by combination with PsIA12 or R39 on sandy loam (table 4).

The *Pseudomonas* strain PsIA12 improved the effectiveness of a *Rhizobium* inoculation of alfalfa on loamy sand and sandy loam (table 4). In comparison to a single inoculation with *Rhizobium*, seedling development was accelerated, the raw protein content and the uptake of P and K were increased, and the root development in deeper soil layers (40–60 cm) was promoted about 25% (ref. 50).

The seed yield of maize on loamy sand was repeatedly increased in field experiments with AM fungi (table 1). So far, positive effects of combined inoculations with R39 and PsIA12 have only been found in pot experiments. *Azospirillum* spp. *Azotobacter croococcum*, *Bacillus polymyxa*, and *Pseudomonas stricta* equally increased the colonization of *Pennisetum padicillatum* with AM fungi[67]. No advantages of the combination AM fungi/*Pseudomonas putida* could be observed in *Medicago sativa* and *Lotus corniculatus*[64].

Prerequisites for positive inoculation effects under field conditions

The results obtained so far have shown that plant growth can be stimulated by inoculation with phytoeffective microorganisms. Since these microorganisms live in a very close association with the plants, their effectiveness is more dependent on ecological factors than that of fertilizers and plant protection products. The reason for plant growth promotion not being satisfactorily reproducible may be:

- insufficient strain screening for the actual site of cultivation;
- the loss of advantageous physiological characteristics of the strains during cultivation in the laboratory;
- the inoculum form and method of inoculation not being optimal for survival during storage and for establishment in the rhizosphere;
- insufficient root exudation of the plant due to unfavourable growth conditions.

The preconditions for positive inoculation effects are:

- efficient symbiotic or associative microorganisms;
- suitable inoculum types and methods of inoculation, and
- suitable growth conditions for an effective symbiosis between micro- and macrosymbiont during the growth period.

Selection of growth-stimulating microorganisms

The large variety of genetic forms provides the possibility of selecting competitive strains with advantageous metabolic and plant colonization characteristics from the natural microorganism populations (see above 'Materials and methods' and 'Importance of microbial metabolic performance. . .'). Effective microorganisms must be able to survive in the rhizosphere during the vegetation period. It is therefore necessary to test plant-specific growth-stimulating effects found in laboratory or pot experiments for reproducibility under specific field conditions. This requires that the efficient characteristics of the microorganisms must remain stable during the cultivation of the cells for the inoculum.

Suitable types of inoculum and methods of inoculation

The type of the inoculum and the method of inoculation can influence the survival and the efficiency of microorganisms. Preparations on a peat basis ($10^8 - 10^9$ cfu/g peat) have repeatedly proved to be suitable for our symbiotic and associative bacteria[34,42]. The AM fungi inoculum produced on the basis of a peat-bentonite mixture (3:1) with maize as host plant was suitable for seed inoculation and, in the form of granules, for seed row inoculation[33,38]. The two types of inoculum can be combined. The amounts necessary for bacterial inoculation are lower (0.4–1.0 kg/hectare) than those for AM fungi inoculations (20–40 kg/hectare). A titer of 10^5 to 10^6 cfu per seed has proved to be suitable; it can be lower in the case of effective bacteria. Positive effects have also been achieved with other carriers, e.g. coal, compost, polyacrylamide gel[15,62].

The type of inoculum and method of inoculation must be generally adapted to the aim and to the conditions of use. Their concrete application will be influenced by economic considerations.

Growth conditions necessary for positive inoculation effects at the site

Since microorganisms stimulate plant growth in nutrient-rich as well as nutrient-poor sites, applications for different purposes are possible, e.g.:

- for achieving an economic yield increase in the plant production, or
- for a biological reactivation of devastated soils.

The plant producer can favour positive inoculation effects by observing the general principles of crop production, for instance by ensuring that the host plants are appropriately spaced to avoid the growth of large pest populations, and by establishing conditions favouring an accelerated seedling development. These include optimal seed-bed preparation, the establishment of pH values optimal for the crop species by liming, and ensuring a sufficient supply—at least to the seedlings—of the basic nutrients. On nutrient-poor loamy sand, an additional P fertilization can favour the effect of AM fungi on maize (unpublished). The amount of fertilizer necessary for an effective inoculation must be determined for the particular site and crop species in accordance with the aim of the treatment. For instance, no nitrogen fertilizer is necessary when legumes are inoculated with efficient *Rhizobium* bacteria. In addition, recommendations for the application of plant protection products should be observed, in order to prevent phytotoxic effects.

Crop damage caused by weather extremes (e.g. drought) or heavy pest infestation can, as a rule, not be compensated by inoculations with microogranisms.

The biological reactivation of devastated soils demands plant and microorganism populations which are adapted to the specific conditions of the site. They must be selected to be specific for the site and the purpose, and tested for effectiveness and stability of performance. Again, the specific use demands investigations to define appropriate methods of inoculation and to find possibilities of improving plant development by the appropriate measures of cultivation.

Conclusion

A stimulation of plant growth under field conditions in temperate climates is possible for various crop species (legumes, gramineae, cruciferae) on different types of soil by inoculation with selected, efficient microorganisms. Depending on the crop species, the degree of growth stimulation can be modified by soil, climate, and pests.

Positive effects were achieved by inoculations with selected *Rhizobium* bacteria, AM fungi and associative bacteria, especially *Pseudomonas* spp. Some isolates of growth-stimulating microorganisms possess several phytoeffective capacities (N_2-fixation, P-mobilization, phytohormone production, antagonistic effects). In some cases, the effect of single inoculations can be improved by inoculating microorganisms with different metabolic and colonization capacities. The effectiveness of the inoculation must repeatedly be proved for the specific crops under field conditions; the survival of the microorganisms in the rhizosphere during the growth period is of special importance. Not only *Rhizobium* bacteria and AM fungi but also associative bacteria have this capacity. Effective associative bacteria partly colonize the intercellular spaces of the root, and important growth centres such as the mucigel of the root tip and the cortex ruptures resulting from emerging lateral roots.

In young plants the characteristics of effective inocula were especially an enhancement of root length and branching and of shoot and root dry mass, as well as better protection against soil-borne plant pathogens. Effects such as increased nodulation and AM fungi colonization by organisms from the wild population were partly brought about by the inoculation. Appropriate inoculum types and methods of inoculation provide the possibility of favouring quick root colonization and the survival of the microorganisms, even in temporary stress situations in the soil. Since the production of phytoeffective microbial metabolites depends on the supply of plant assimilates, the observance of efficient cultivation methods, for instance crop rotation, seed bed preparation and basic fertilization, can improve plant development and thus the effect of the inoculation.

Research is still necessary to analyse the interactions between microorganisms and plants, for instance with regard to the effects of phytohormones; competition in root colonization; biological, chemical, and physical factors of soil and climate, and their importance for the growth of plants. The utilization of selected growth-stimulating microorganisms is, however, not bound to the complete elucidation of all fundamental principles. This is demonstrated by the fact that *Rhizobium* inocula are already in use in many countries.

The application of microbiological inocula can be extended further in plant production and also used for the biological reactivation of devastated soils. For this purpose it is necessary to select and test microorganisms in close association with site-specific plant commuities, to elaborate appropriate inoculation methods, and to optimize the conditions for the development of the plants.

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