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Review article

What can be Learnt From the Current Use of Inoculants in Legume Production? The Relative Merits of Mineral Fertilisers and N-Inoculants

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Abstract

The overall input of nitrogen into global agriculture for food and feed production is estimated to be approximately 120 million tonnes/yr. Biological nitrogen fixation (BNF) accounts for 40 million tonnes/yr and 80 million tonnes/yr is accounted for by N-fertiliser production from ammonia. In cereal production fertiliser use dominates. If cereals were able to "fix" their own nitrogen the situation could be very different. However, this is unlikely to be realised in the near future unless the technological complexities of inducing BNF in non-legume crops can be overcome. Traditionally, work in this area has tended to focus on the transfer of legume-like BNF characteristics to non-legumes and so far commercially, this strategy has not been successful. More promising may be work that has purported to show that some

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species of endophytic bacteria living within non-legumes (e.g. grasses) can supply nitrogen to their host plants. The potential of this approach is discussed here based upon current experience with the use of bacterial inoculants for legume production (in particular Soya production). The relative merits of N-inoculants and N-fertilisers are discussed along with issues that are relevant for the future development of BNF in non-legumes as seen from an industrial perspective. Many of the current environmental concerns about the use of mineral fertilisers can also be applied to the use of N-inoculants. It is concluded that if N-inoculants for non-legume crops are developed then these will have to be at least as convenient, safe, reliable and effective in growing crop for an ever-increasing global population as N-fertilisers are today.

Keywords: Fertilisers, nitrogen, non-legumes, microorganisms, endophytes

1. Introduction

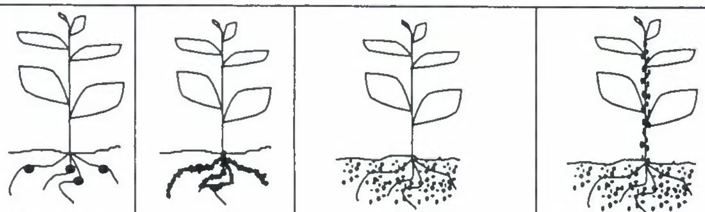
The use of commercially available bacterial inoculants (N-inoculants) is reserved largely for the production of Soya and other pulses. In principle, similar products could be developed for application on non-legume crops and over the years there have been many reports of research that has attempted to show the utility of such an approach (Andrews et al., 2003). Regardless of the reasons for wanting to develop such products, more broadly applied N-inoculants will have to at least match the performance of both mineral N-fertilisers and organic sources of nitrogen (e.g. manure) in providing food and fibre required to feed and clothe a growing global population. The relative merits of N-inoculants and N-fertilisers are reviewed and this is used as a basis and guide as to what features might be required in the future for commercial N-inoculants for non-legumes, particularly cereals. Whether or not N-fertiliser application can be directly supplemented by the use of inoculants is discussed, and the potential role of endophytes as model future inoculants is examined. Finally, some advantages and disadvantages of the application of N-inoculants are considered.

2. Microorganisms as Inoculants

Nitrogen fixing microorganisms receive carbon or other sources of energy from their association with plants. The latter receive nitrogen and/or other growth promoting substances (e.g. hormones) in return. Some microorganisms, such as *Azospirillum*, fix nitrogen for their own benefit whilst supplying plant growth promoting hormones to their host plant. BNF can be classified according to the type of association that exits between the microorganism and the host plant:

- *Symbiotic nitrogen fixation*: Microorganisms (*Rhizobium* spp.) fix nitrogen from the atmosphere after infecting the cortical cells of roots and eliciting the formation of root nodules – this phenomenon is unique to legumes. The principal nitrogen-fixing systems useful in world agriculture are found in legumes such as peas, beans, soybeans, chickpeas, lupins and Alfalfa. All involve association of plants with rhizobia of which *Rhizobium* and *Bradyrhizobium* are the most well known examples. Rhizobia are commercially exploited as inoculants for soybean production in the USA, Brazil and Argentina.
- *Associative nitrogen fixation*: Microorganisms fix nitrogen from the air while inhabiting either the rhizosphere or the outer layers of the root surface. In the soil there is also the possibility that "free-living" diazotrophs can fix and contribute nitrogen to the plant though not necessarily in close plant-microbe association.

Yet another classification of plant microbe interactions involves endophytic bacteria. These live within plant tissues, in stems and leaves but not within plant cells. Nitrogen is fixed from the atmosphere without the aid of plant root nodules. In Brazil it was claimed that some species of sugar cane grow naturally over many years without yield loss due to inputs of nitrogen supplied in part by endophytic bacteria (Boddey et al., 1995). The major strategies of land-based nitrogen fixation are illustrated in Fig. 1 (after Marschner 1995).



System of N_2 fixation ($N_2 \rightarrow NH_3$) and Microorganisms involved	Symbiosis (eg Rhizobium, Actinomycetes)	Associations (eg. *Azospirillum, Azobacter*)	Free living (eg. *Azobacter, Klebsiella Rhodospirillum*)	Endophyte bacteria (eg. *Azobacter, Herbaspirillum, Glucanurobacter*
Carbon energy source	Sucrose and its metabolites (from host plant)	Root exudates from the host plant	Heterotroph: Plant residues Autroph: Photosynthesis	Sucrose & its metabolites from the host plant

Figure 1. Type and energy source of biologically nitrogen fixing systems in soils. Source: modified after Marschner (1995).

Table 1. Cereal and legume (pulses and soybeans) production in different world areas in 2002

	Cereals		Legumes[1] Pulses		Soybeans	
	Area (000 ha)	Production (000 Mt)	Area (000 ha)	Production (000 Mt)	Area (000 ha)	Production (000 Mt)
Asia	163,492	446,186	24,425	15,752	6,090	7,996
Africa	35,183	65,530	7,200	4,070	965	921
Australia	15,766	18,931	2,054	1,696	33	70
Canada	15,509	34,868	2,080	2,587	971	2,255
China	82,083	401,808	3,157	4,374	9,420	16,900
E. Europe	52,403	145,710	2,333	2,973	311	543
EU-15	37,665	214,459	1,633	4,501	296	731
Mexico	10,897	29,114	2,035	1,677	76	127
Russian Fed.	41,664	84,375	697	1,243	390	360
S. America	31,075	94,479	4,701	3,642	27,773	72,192
USA	54,306	299,211	877	1,593	29,056	73,201
Rest	123,497	197,078	16,423	10,063	3,786	4,680
World	663,540	2,031,749	67,615	54,171	79,167	179,976

Asia: Bangladesh, Cambodia, India, Indonesia, Japan, Pakistan, Philippines, Thailand and Vietnam. Africa: Algeria, Egypt, Kenya, Nigeria, South Africa, Uganda, Zambia and Zimbabwe. Eastern Europe: Bulgaria, Czech Republic, Fed. Rep. of Yugoslavia, Hungary, Poland, Romania, Slovakia, Turkey and Ukraine. EU-15: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Italy, Ireland, Luxembourg, Netherlands, Portugal, Spain, Sweden and United Kingdom. South America: Argentina, Brazil, Chile, Colombia, Uruguay and Venezuela. [1]In addition, the production of forage legumes accounts for ca. 600 million metric tons (Mt). Source: data compiled from FAO, Statistical database FAOSTAT (http://www.fao.org).

3. Food Production, Inoculants, and N-Fertilisers

Not all plant-microbe interactions lead to the transfer of nitrogen to the plant. However, when transfer does occur, for example, in legumes, rice and in sugar cane, the amounts of biologically produced nitrogen taken up by the plant can be sufficient for normal growth (though not necessarily high yields). In food production, plants utilising BNF contribute in three principal ways:

- As a direct use as food, e.g. peas, beans, protein from Soya, sugar from sugar cane
- For conversion to meat via cattle fodder, e.g. Alfalfa, clover, soybean supplied as cakes, forage or silage

- As a source of Green Manure, e.g. clovers, Melilot and Alfalfa are ploughed in to upgrade poor or spent agricultural land.

Though some species of sugar cane are able to fix their "own" nitrogen via endophyte activity, the majority of commercially grown sugar cane is produced using fertilisers. Rice is also grown mainly using nitrogen supplied either from the soil, manure or from urea fertilisers. A comparison of cereal and legume production gives a measure of the significance of BNF to global food production. As shown in Table 1, the total production of pulses and soybeans combined is much less than the total production of cereals, (if forage legumes are taken into account the production of cereals is approximately two fold greater than the production of legumes). If the total contribution of land based BNF to the global N-cycle is considered, the amount of N-fixation is relatively high:

100–200 million ton N/yr (Newton, 1996)
90–140 million ton N/yr (Lægreid et al., 1999)
140–180 million ton N/yr (Sococlow, 1999 from Schlesinger, 1997).

If agricultural production alone is considered, the above figures for the contribution of BNF fall to approximately 40 million tons N/yr (Galloway et al., 1995). Smil (2001) has recently suggested a figure of approximately 30–35 million ton N/yr for "agricultural biofixation". Soybean is the largest commercial crop that makes use of biological nitrogen from rhizobia present naturally in the soil or rhizobia formulated and applied as commercial N-inoculants. The amount of nitrogen supplied in the form of fertilisers is easier to determine; approximately 80 million ton N/yr as ammonia for N-fertiliser production is currently produced and applied in agriculture (Smil, 2001). On a global basis, the two main crops benefiting from the application of N-fertilisers are wheat and rice. The principle challenge is to continue to increase food production to match the demands of an increasing global population, whilst at the same time limit any negative environmental effects (Lægreid et al., 1999). As the global population increases, the amount of land available for growing food is diminishing and this presents a further challenge (Fig. 2).

On a global basis, the energy consumed to produce ammonia, the basic N-fertiliser building block, is small compared to the benefits obtained from fertilisers in terms of food and feed output. Modern efficient fertilizer plants use relatively little energy. The generation of greenhouse gases is low and will become lower as technology reduces the output of nitrous oxide and carbon dioxide, byproducts of the Haber Bosch process. In a detailed examination of the energy consumption and greenhouse gas emissions in fertilizer production, Kongshaug (1998) has estimated that modern fertilizer production consumes approximately 1.2% of the world's energy and is responsible for approximately 1.2% of the total emission of greenhouse gases. More recently, Smil (2001) has

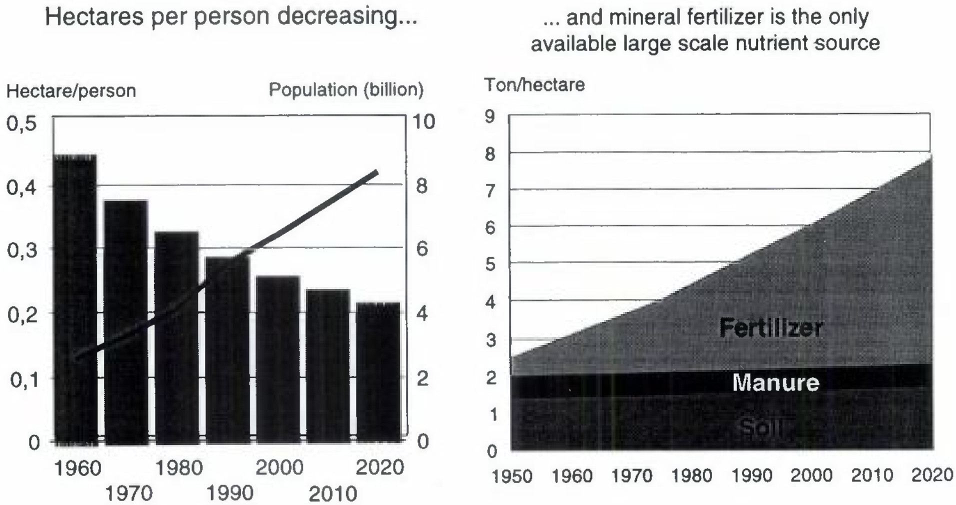


Figure 2. Plant nutrients are required to produce more food from less arable land. Source: IFA, Worldmarkets.com, PCS, Hydro Agri.

estimated that the energy needed to fix fertilizer nitrogen amounts to 1.3% of all energy derived from fossil fuels. The increased energy crises during the last 25–30 years have caused a positive downward trend both for energy consumption and greenhouse gas emissions within fertiliser production (Kongshaug, 1998).

Fertilisers are applied to crops depending on local soil conditions and practice. Urea (46% N), calcium ammonium nitrate (20–28% N) ammonium nitrate (33–34% N), and compound fertilisers based on N, such as NPK, are among the most commonly applied fertilisers in the world. In 2001–2002, total world consumption of N-fertilisers was estimated to be between 82–86 million tons (IFA Secretariat, 2002; FAO, 2001). Consumption is projected to rise to 9.4 million tons by 2005/6 (FAO, 2001). The use of fertilisers differs widely depending on economic reasons as well as agronomic practice and circumstances. In the fertile wheat fields of mid-west Canada very little N-fertiliser is used whereas in Northern Europe, in France, Germany and the UK, high amounts of N-fertiliser are applied with correspondingly much higher yields than those obtained in North America (Table 3). In parts of Africa not enough fertilisers are applied (as much due to poor transport and logistics as to reasons of farm poverty), and already nutrient impoverished soils are continuing to degrade further.

Crops such as modern varieties of wheat and rice have been bred to respond to fertiliser applications. The limiting nutrient is nitrogen, though nitrogen alone is not sufficient for healthy plant growth and a high yield of product. Other primary and secondary nutrients (P, K, S, Ca, Mg) as well as trace elements (e.g. Cu and Fe) should also be applied appropriately, according to the principles of balanced nutrition and good agricultural practice (Lægreid et al., 1999). Microbial inoculants may have a potential role to play in promoting the uptake by plants of other nutrients besides nitrogen.

4. Inoculants Technology

Inoculants have been sold since the beginning of the 20th century based on fermentation technology that has changed in principle very little over time. Some inoculants companies are listed in Table 2 and these produce and sell microorganisms for the inoculation of legumes and some non-legumes around the world. The microorganisms are formulated as peat mixtures or as powdered or liquid products. Powder inoculants are the simplest and oldest type of product and the microorganisms are simply stuck to the seed as a coating before planting.

The most common example of the commercial use of BNF today involves the use of bacterial inoculants on Soya. The use of inoculants technology is essentially "low tech". Inoculant strains are chosen for their propagation ability, shelf-life, and their ability to survive and operate reproducibly in the field. The fermentation and application technology in use is non-specific and the proprietary value often lies in the knowledge associated with formulation of finished products (e.g. *Rhizobium*-coated seeds). Most of the new inoculation products are derived from improvements in formulation rather than in the use of newly discovered efficient strains of microbes.

The inoculants business is highly competitive and the distinguishing features of the more successful companies are:

- Competitive and high quality production processes and know-how (e.g. composition of the fermentation medium to give high cell density and high survival),
- Good quality product formulations (e.g. a liquid carrier composition to give long shelf-life),
- Pre-inoculation seed technology and continual improvement of the viability and survival of chosen microbial strains in the field.

Commercially, there are few products and these are mainly based on mixtures of free-living bacteria or individual facultative endophyte strains such as *Acetobacter*. Despite claimed benefits in sugar cane and rice, associative nitrogen-fixers as inoculants for non-legumes are not major products of the inoculants industry. Production of legumes is not dependent on fertilisers and commercially the amount of fertilizers sold into this segment is relatively small.

Table 2. A selection of inoculant companies (in alphabetical order)

Agribiotics Canada & USA	Produces high quality rhizobia inoculants for legume production. Products are peat based or liquid formulations. Also interested in general aspects of microbial plant growth inoculants.
Bio-Care Technology Pty Ltd. Australia	Inoculants for creating nodulation and nitrogen fixation in pasture and grain legumes such as Alfalfa, clovers, Lotus, Lablab, vetches, Guar, Leucaena, peas, soybeans, cowpeas, mung beans, lupins, chickpeas, faba beans, lentils, etc. Contains *Bradyrhizbium* spp, *Rhizobium* spp., *Sinorhizobium* spp.
Circle One Georgia, USA	Sells products for sustainable agriculture, a range of soil conditioners, inoculants and biocontrol products. Not only for legumes. They claim effects also on wheat. Products contain enzymes, *Bacillus subtilis, Bacillus ceres, Bacillus thuringensis, Bacillus megaterium, Azotobacter* spp. *Aspergillus oryzae* extract, *Lactobacillus,* seaweed extracts, *Rhizobium* spp.
Lipha Tech	Principal offices and locations are in France, in Milwaukee, US, and in Buenos Aires, Argentina. They have sold inoculants since 1898. The inoculants are *Rhizobium* for soybean, peanuts, Alfalfa and clover, dry beans, peas and lentils. Commercialisation of *Azospirillum* as an inoculant for non-legumes (maize) has been attempted but has not proved so successful.
Nutri-tech Solutions Australia	Manufactures *Azotobacter* as an N-fixer, and a producer of plant hormones. The product is to be applied in irrigation water or as soil injection.
Philom Bios Inc. Canada	Main products based on superior *Rhizobium* strains for legumes. Developed P inoculant for cereals, excellent for direct seeding, no-tillage situations. All aim at high-end of the market. Interested in general plant growth promoting effects of microorganisms.
Star seed Kansas, USA	A seed company that also produces and sells seed inoculants for legumes – soybeans and Alfalfa.
Urbana Labs. MO, USA	Claims to be one of the world leaders in manufacturing and development of *Rhizobium* inoculants for all legume crops such as soybeans, peanuts, peas etc.

5. Can N-Fertilisers be Supplemented by Application of N-Inoculants?

Whether nitrogen is obtained from mineral fertilisers or other sources is immaterial to plants, providing that it is in a form that can be used. In plants, mineral fertilisers help to increase the productive use of solar energy by producing biomass, which in turn is used as energy when consumed as food or as fuel. Inoculants, as applied currently to legumes, perform exactly the same function. A current problem with legume N-inoculants (and presumably endogenous endophytes in rice and sugar cane) is that in the presence of readily available sources of other forms of nitrogen, the biological nitrogen fixation process is inhibited. Understanding this process may help to devise ways around this problem.

Combined nitrogen (in the form of nitrate, ammonium ions, amino acids, and urea) present in the soil will affect the contribution of inoculants to plant growth. This contribution depends largely on the dose of N available, the period at which it is available, and its actual form. For symbiotic systems, increasing N availability from the soil will first enhance the total contribution of biological sources of nitrogen and then reduce it. At an early stage in legume production, low doses of N (generally below 30 kg N/ha) stimulate subsequent nitrogen fixation by promoting the establishment of nodules. The N here sustains the lag phase period between root infection and the beginning of nitrogen fixation activity by enabling the plant to produce sufficient level of assimilates to sustain the nodule growth and activity. At a higher level, nitrogen seems to inhibit the symbiotic processes. As a consequence, nitrogenase activity is decreased as well as the number of nodules per plants. According to Marschner (1995) nitrate is generally found to decrease either the nodulation processes or nitrogenase activity, or both. One inhibiting effect on nodulation seems linked with the way infection occurs. When infection occurs via root hairs, high levels of nitrate will have an indirect effect as it decreases root hair density. Species for which infection occurs via other pathways (such as at sites of lateral roots) will not be so sensitive to high nitrate concentrations (Kohls and Barker, 1989). But nitrate can also affect activity of the nodule itself and of the nitrogenase enzyme. Here, sensitivity varies between species (e.g. Soybean>>Lupine), and even between cultivars. It has also been shown that by improving the overall level of soil fertility and particularly the P content of the soil, the inhibiting effect of nitrate can be alleviated (Tsai et al., 1993). It seems that the N:P ratio here is the determining factor (Huss-Danell et al., 2002), but more explanations on the mechanisms involved are required. Placement of the nitrate source is also important and it has been shown that in pot experiments a continuous supply of low-level nitrate (1 mM nitrate) from the lower roots can promote nodulation and nitrogen fixation in the upper part of Soybean (Yashima et al., 2002).

Of the several explanations for the inhibiting effect of nitrate in legumes, two factors might apply also to endophyte-plant associations:

- Competition for carbohydrates at the root level, especially when nitrate is reduced in the roots of the host legume, can inhibit nodule formation and/or function since root N-assimilation takes precedence. (Availability of an

organic source of carbon is a factor in determining nitrogen fixation in endophyte-plant associations).
- Feedback regulation, due to a high content of reduced N compounds in the phloem sap of the host plant that also feeds the nodules, down-regulates nodule formation and /or function. (Feedback regulation in endophyte-plant associations might also be expected to control the rate and amount of N-fixation in these systems).

In practice, except for an early and low N supply (starter effect), the use of N fertilizer is not recommended if the farmer wants to maximise N nutrition via BNF. This is particularly true for soybean, where little or no N is currently added in Brazil and in some areas of the USA. In pastures, if the soil N content increases as a result of N-fertiliser application, legumes will disappear rapidly. This is partially explained by the fact that under high N availability, the grasses out-compete the legumes for growth. But even with no N added, it has been observed in a cycle lasting four years that legumes are first dominant and then decline gradually as soil N content increases, but reappear again once the grasses have exhausted the inorganic N pool in the soil (Ledgard and Steele, 1992).

High soil nitrogen (especially nitrate), drought, low/high root temperatures and soil acidity are deterrents to biological nitrogen fixation in legumes and similar effects might be expected for non-legumes. Little is known about the interaction of N with other limiting factors such as P, and also on the effect of the timing of N supply. Additional nitrogen though does not always lead to a reduction in BNF. On the contrary, it has been reported that soybean yield was substantially increased by three split applications of N (25 kg as starter N, 50 kg at flowering or early vegetative growth and 25 kg as top dressing after flowering), while the percentage of N coming from BNF (84%) remained unchanged at harvest (van Kessel and Hartly, 2000, after a study of Yinbo et al., 1997). The hypothesis is that after flowering, the sink strength of the seeds is so high that there is no negative feedback regulation from additional N supply. Thus there might be better ways to combine N fertilizers with nitrogen fixation for optimising legume yields that may be applicable also to non-legume systems.

The hope is that with the application of modern biotechnology breakthroughs will occur leading to the commercial reality of cereal plants able to fix their own nitrogen. This objective is still long-term despite some recent very encouraging results in peas and alfalfa that has identified a receptor protein molecule that enables bacteria to colonise the host plant (Stracke et al., 2002). The gene encoding this molecule, called NORK, has also been identified and apparently is identical to the receptor called SYMAK, critical for symbiosis between plants and mycorrhizae fungi (Endre et al., 2002).

The dream among some researchers has always been to enable or extend the nitrogen fixing capabilities of legumes to non-legumes and such discoveries may make this possibility more of a reality in the future. However, these discoveries also serve to highlight the complexity of the process. Until this is resolved there are other ways perhaps of achieving BNF in non-legumes that are perhaps more interesting, in particular the recently published work with endophytes and cereals.

6. Endophytic Bacteria as Models for Future N-Inoculants

BNF in non-legumes has traditionally focused on the production of "green manures" or "biofertilisers" for agricultural use. For example *Azolla*, an aquatic fern is grown to provide a nitrogen source in subsistence agriculture in Asia. More interesting though are the many observations and reports of the joint culturing of endophytic and/or free-living bacteria associated with rice roots. Endophyte bacteria have the ability to survive in plant tissue in sugar cane, wheat and rice, but as yet have not been commercialised. These bacteria exist naturally in some species of sugar cane and in rice and have become targets for experimentation.

Recently, Riggs et al. (2002) have published work that promises "to increase wheat and/or rice growth in the absence of added fixed nitrogen" (mineral fertilisers). The results so far appear to demonstrate that *K. pneumoniae* 342 survives endophytically in wheat and that in pot experiments, total N per plant and dry shoot weight increased relative to uninoculated plants, and to control plants that received water alone. In rice, beneficial effects were found using *G. diazotrophicus* PA15 (from sugar cane) and *Pantoea* (from switchgrass). Compared to the uninoculated control, *Pantoea* increased dry weight by 57% and total N/plant by 72%, and *G. diazotrophicus* increased dry weight and total N/plant by 55%. What remains unclear is whether or not these results reflect real nitrogen fixation by endophytes within the plants in question. Isotopic labeling studies using N^{15} are already planned to establish whether plant growth is directly attributable to endophyte-supplied nitrogen (Triplett, 2002). In addition to field studies, further experiments are required to confirm the bacterial nitrogen fixation *in planta*.

Peoples et al. (2002) have recently summarised data from a number of studies carried out between 1992–2000 and conclude that the amount of nitrogen commonly observed as being fixed by associative and free-living microorganisms is 10–65 kg shoot N/ha and 0–15 kg shoot N/ha, respectively, compared to symbiotic systems (legumes) that fix between 30–300 kg shoot N/ha. Measurements quoted in the general literature usually fall within a broad range of 10–100 kg N/ha per crop for non-legume systems and 0–680 kg

N/ha for legumes (as a comparison, corresponding figures for fertiliser use on wheat are shown in Table 3).

Sugar cane has been listed as among the top ten crops with the highest N-fertilizer application rates of up to 308 kg/ha (IFA, 1999). On average, N-fertilisers are applied to 94% of the commercial sugar cane crop though in Brazil the amount of N applied as fertiliser is often much less than that removed from the field when the crop is harvested (Boddey et al., 1995, 1995a). This is done in poor soils and conclusion is that these varieties of sugar cane benefit from "large contributions of plant-associated BNF". Under controlled conditions in pot experiments the canes have been reported to remove up to 150 kg N/ha (Boddey et al., 1995a). Further, it has been claimed that all the nitrogen needs of the crop can be satisfied using endophytic bacteria. The plants live in association with *Acetobacter* and *Herbaspirillum* but it is not proven by re-inoculation studies that these species are able to supply the N required for plant growth. The bacteria can survive unusual conditions for growth (>30% sucrose in the growth medium) and are often very slow growers *in vitro*. Recently, Lee et al. (2000) have sequenced and analysed the entire nitrogen-fixing gene cluster in *Acetobacter* – the cluster represents the largest nitrogen-fixing genes characterised so far in any diazotrophic bacterial species. Further studies are currently required to estimate accurately the nitrogen fixing ability of this species in sugar cane.

In the tropics, lowland rice yields of 2–3.5 ton/ha are normally achieved using naturally available N derived from BNF by free-living and plant associative diazotrophs (Ladha et al., 1993, 1997) and from mineralisation of soil N (Kundu and Ladha, 1995). Growth may also be stimulated by *Rhizobium*, but rice does not form nodules and any growth is not a consequence of BNF from this source. Much higher yields (up to ca. 6–8 ton/ha) are obtained in those

Table 3. Average application rates of N-fertilisers on wheat and average yields

Country	Area (000 ha)	Average fertiliser rates (Kg/ha)			Average yield (T/ha)
		N	P_2O^5	K_2O	
EU – North	9166	136	30	40	7.0
EU – South[1]	8199	85	43	18	2.6
USA[2]	29157	59	34	28	2.7
Canada	12626	45	25	5	2.1

[1]EU – South: 1/2 France, Greece, Italy, Portugal, Spain; [2]USA – N range = 43–75 kg/N.
Source: Data compiled from IFA/IFDC/FAO (1999) and Harris (1998).

areas where mineral fertilisers are used. Lowland or "wetland" rice is grown using urea as the major source of mineral fertilizer. Rice is grown in an environment conducive to N losses via denitrification, ammonia volatilisation, leaching and runoff of applied fertilizer that affects the N-use efficiency that can be achieved. The amount of N applied therefore, depends on local circumstances, but on average falls between 75–125 kg/ha (Bøkman, 1997; IFA, 1999). Korea, China and other countries apply more fertilizer than does the USA, though the yields (ca. 6.0–6.3 ton/ha) are very similar (Harris, 1998). If endophytes in rice were able to supply as much as 150 kg N/ha (as required by sugar cane, according to Boddey et al. (1995a)), then yields of rice higher that 2.5–3 ton/ha might be entirely possible. In comparison, Peoples et al. (2002) suggest that legumes should routinely be fixing at least 100 kg N/ha/year.

7. Nutrient Recycling between Bacteria and Plants

There is considerable speculation on the involvement of bacteria on nutrient recycling in the rhizosphere. There are research groups around the world that aim to develop *Azospirillum* and other associative bacteria as inoculants with a wider host range than just maize. It has been clearly demonstrated that the plant growth promoting effects of *Azospirillum* are due to the production of auxin and are not due to the transfer of nitrogen to the host plant. This opens up possibilities of using microbial inoculants to promote or stimulate nutrient uptake in plants. Unfortunately, precise mechanisms of the interchange of plant nutrients and other substances, including particular signalling substances, that promote fruitful host plant-bacteria associations, are presently often unknown or poorly understood.

8. Advantages and Disadvantages of Endophytes as N-Inoculants

Likely advantages of using endophytes as N-inoculants from an industrial perspective are:

- Unlike free-living microorganisms, the bacteria reside in the stem and leaves and not in the roots or grain and are therefore not likely to "escape" into the soil or remain upon harvest (unless the crop residues are intentionally re-incorporated into the soil). The introduction of microbiological products will be subject to scrutiny and the perceived safety or otherwise of their use will be questioned by government regulatory bodies and non-governmental organisations alike.
- Inoculation is required every year causing repeat business (as with fertilizers). Commercially this is an important aspect that needs to be considered.

- N-Inoculants are likely to be crop specific (different products for rice and wheat in the first instance). The farmer will be offered specific products tailored to the needs of the crop (and variety?).
- Conventional production and formulation technology (i.e. fermentation and seed-coating) can be readily applied. The aim would be to emulate the success of the high quality *Rhizobium* based inoculants that are sold today.

The disadvantages include stability, handling and safety issues that arise in using living organisms. Viability and reproducibility of effect (e.g. nitrogen fixing capability) are important factors. Based on current published results relatively high numbers of bacterial cells within plant tissue are required for effect (4000–6000 cells per gram fresh eight of tissue) (Riggs et al., 2002). Cereal farmers will have to become familiar with handling and applying a "living" product. Other concerns are:

- At present there is concern about the environmental effects of over-supply of nitrogen to fields, causing problems such as eutrophication in rivers and lakes, and escape to the atmosphere of nitrous oxide, a potent greenhouse gas. In principle, nitrogen derived from the widespread use of endophytic bacteria could also become an environmental problem if it is not monitored and controlled. Nitrogen remaining in the crop residues will behave in exactly the same way as nitrogen derived from mineral or organic fertilisers (e.g. manure).
- For optimum plant growth and optimum yield, modern agronomic practice favours a balanced nutritional approach (Lægreid et al., 1999). Supply of N is not enough. It is important that throughout the growing season crops receive adequate amounts of the primary nutrients, N, P, K, and secondary nutrients, Ca, Mg, and S, and sufficient micronutrients or trace elements (e.g. Cu, Fe, Zn, etc.). N-inoculants will have to be formulated and applied with these constraints in mind, particularly if the inoculants are to be applied in areas where already today there is a shortage of some essential nutrients (e.g. in Africa and in parts of South America). Modern fertilisers in contrast, offer a wide range of formulations that enable farmers to apply the correct amount of nutrients conveniently and readily as and when required. If N-inoculants for non-legumes became a reality, it is very likely that nutrient supplements would have to be applied to the growing crops.
- Finally, mineral and organic fertilisers are required to feed a growing population. The ability of N-inoculants to provide the high yields necessary to sustain the amount of food required in the future must be questioned. It may be that in order to maintain high yields in wheat (Table 3), or in rice (Table 4), N-fertilisers will still have to be used. Whether N-inoculants could be used in the presence of N-fertilisers remains technically open to question, as has been discussed.

Table 4. N fertilizer application related to rice yield in selected countries

Country	Total fertiliser applied (kg/ha)	Total N applied (kg/ha)	Yield (ton/ha)
China and USA	223–184	147–145	6.27–6.02
Indonesia	117	90	4.34
India, Bangladesh and Brazil	81–59	54–17	2.88–2.48
Thailand and Vietnam	54–26	38–26	3.64–1.37
Madagascar	3	1	2.13

Source: Data compiled from Harris (1998).

9. Conclusion

The ability of bacteria to inhabit not only plant roots but also whole plants appears to be an attractive property if coupled with the ability of the same bacteria to transfer nitrogen to the host plant. If these bacteria could be induced to populate wheat and rice plants from seedlings then inoculants could presumably be formulated in much the same way as inoculants for soybean are formulated today. The challenge for the immediate future then is to test out the ability of different strains of endophyte to invade and colonise host plants such as wheat, rice and maize. The next step is to demonstrate beyond doubt that the bacteria present in the plant tissue can truly support plant growth by fixing nitrogen. However, if N-inoculants are to be developed in non-legumes then these will have to be commercially competitive and perform as well as mineral fertilisers. In practice, N-inoculants will have to prove to be as convenient, safe, reliable and effective in growing crops for an ever-increasing global population as N-fertilisers are today.

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