CHAPTER 11

Soil microorganisms:

Key players in crop nutrition on the prairies

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ABSTRACT

Soil microorganisms have a tremendous impact on crop productivity because they influence, directly or indirectly, the health of plant roots. The ecology of microorganisms in the rhizosphere affects nutrient and moisture availability to the plant and the incidence of soil-borne diseases, the three factors which most restrict optimal crop yields under western Canadian growing conditions. Microorganisms can increase the nutrient status of the soil by direct addition (e.g., N_2 fixation), solubilization (e.g., P and S) or chelation (e.g., Fe) of nutrients required by the plant. Microorganisms can exude plant growth regulators which stimulate root and plant development. Microorganisms can also alter the sensitive balance of root disease.

The biological approach to soil fertility is in its infancy because of our lack of knowledge of soil microbial ecology and due to the minimal emphasis placed on understanding the plant root as an important component of crop physiology. The few commercial microbial products which exist to manipulate the plant rhizosphere are very successful. Rhizobial legume inoculants can result in fixation of over 100 kg N ha^{-1}. Phosphorus-solubilizing fungi and biological control agents are also beginning to have an economic impact. The eventual success of microorganisms as agronomic inputs to enhance crop productivity will depend on the effectiveness and consistency of their agronomic performance, the ease of the inoculation procedure and their cost-effectiveness and will be much less influenced by any desire to replace traditional chemical inputs.

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CONTENTS

INTRODUCTION

Soil microorganisms are perhaps the least understood but arguably the most important of any edaphic factor that determines crop productivity because soil microorganisms control both the availability of inorganic and organic nutrients, and the incidence of crop disease.

Soil microbiology remains a primitive science and thus we are limited in our ability to manipulate the impact of soil microorganisms on crop productivity. We can isolate and identify only a few genera of soil microorganisms. We understand the role of some microorganisms in the key nutrient cycles in soils (C, N, P, S) but do not understand their ecology and have been remarkably unsuccessful in reintroducing those few known microorganisms back into their natural ecosystem.

This paper will focus on the **active** role of microorganisms in directly or indirectly promoting plant growth. Other chapters will discuss the result of general microbial activity (soil biochemistry) in the N, C, P and S cycles.

MICROORGANISMS AND PLANT GROWTH

The Rhizosphere

The rhizosphere (Hiltner, 1904) is the area of soil under the chemical influence of the plant root. It is not a physically definable area, nor is it uniform in activity along the root surface (Rovira, 1991). However, it is largely in the rhizosphere where micro-organisms have the greatest direct influence on crop productivity.

The ecology of the rhizosphere is complex because it is not just the micro-organisms, the host plant root or the edaphic environment which determines the effects on plant growth. Microorganisms should be viewed as the biological equivalent of micronutrients exerting beneficial or harmful effects on plant growth depending on their numbers and activities at any given point of time and physical location. A critical point is

that rhizosphere associations and their effect are determined by the genetic control of both the plant and the microorganisms (Holl, 1983; Phillips, 1991)

Modes of Action

Plant growth-promoting rhizobacteria (PGPRs) (Kloepper and Schroth, 1978) are bacteria which:

(1) Colonize the plant root surface

(2) Grow and multiply on the plant root surface

(3) Have a direct or indirect beneficial effect on plant productivity.

Using this definition, PGPRs would include microorganisms which influence plant growth by one or more of the modes of action shown in Table 1.

These microorganisms are active in both the rhizosphere and non-rhizosphere but their concentration is 10 000 to 1 000 000 times greater in the rhizosphere due to the large amount of carbonaceous material exuded by growing and dying roots (Rovira, 1991). It has been estimated that up to 40% of a plant's photosynthate is exuded into the rhizosphere (Atkins, 1991).

Nutrient Utilization Efficiency

Microorganisms are aggressive and effective competitors for available plant nutrients. Average FUE of plants for N is <40%, P <20% and S <10% in part because microorganisms out-compete plants for and utilize these nutrients for their own growth and multiplication.

Microorganisms are known to exude a wide variety of acids (eg., lactic, oxalic, succinic, citric, gluconic, malonic, tartaric) which will reduce soil pH thus solubilizing calcium phosphates (Kucey et al., 1989). Microorganisms degrade organic material for C and energy thus releasing key macronutrients such as N, P and S into the environment. A

Table 1. Modes of action of plant growth-promoting rhizobacteria.

Mode of Action	Examples	Microorganism
Nutrient addition	N_2 fixation-legumes	*Rhiozobaceae*
	N_2 Fixation-non legumes	*Azosprillum*
		Azotobacter
		Bacillus
		Pseudomonas
Nutrient availability	Sulphur oxidation	*Thiobacillus*
	Sulphur reduction	*Desulphovibrio*
	NH_4 oxidation	*Nitrosomonas*
	NO_2 oxidation	*Nitrobacter*
	NO_3 reduction	Various
	P solubilization	VAM
		Penicillium
	Immobilization	Various
Plant growth regulators	Auxins, gibberellins,	*Pseudomonas*
	Indole-acetic acid(IAA) etc	*Enterobacter*
		Serratia
		Bacillus
Cellulose degradation	Pesticide degradation	Various
	Nutrient mineralization	
Disease	Damping Off	*Phythium*
		Rhizoctonia
	Black leg (canola)	*Leptosphaeria*
	Root rot (wheat)	*Cochliobus*
	(canola)	*Fusarium*
	Crown galls	*Agrobacterium*
Disease supression	See above diseases	*P. fluorescens*
		P. putida
		Trichoderma
Ice nucleation	Higher frost tolerance	*P syringae*

large amount of plant available N (up to 70 kg N ha^{-1} in the black soil zone) is due to microbial mineralization of organic materials (Paul, 1969, 1975).

Vesicular-arbuscular mycorrhizae (VAM) colonize roots of most plants (except *Brassica spp*) greatly increasing the root's surface area, and exuding acids which make calcium phosphates more plant available (Kucey et al., 1989).

Denitrifying bacteria reduce NO^-_3 to N_2, resulting in a net loss of N from the soil under high energy (i.e., C) and low oxygen (i.e., reductive) conditions (Aulakh and Rennie, 1984, 1985, 1986, 1987).

A quick overview of the C, N, P and S cycles in soils shows that microorganisms are intimately involved in all phases of plant nutrient availability and are in competition with plants for the small amount of available plant nutrients found in the soil.

Nutrient Addition

Dinitrogen (N_2) fixation-legumes

The best known PGPRs are the Family *Rhiozbaceae* which form a symbiotic relationship with legumes to reduce atmospheric N_2 to NH_3. N_2 fixation is energy-expensive for the plant (32 moles ATP/mole N_2 reduced which the plant supplies as carbohydrate from photosynthesis). Up to 30% of the legume's photosynthate is used to power N_2 fixation in the root nodules. However, N_2 fixation is no more expensive to the plant than reducing NO^-_3 and assimilating NH^+_4 across the cell membranes of the root (Atkins, 1991). Plants in N_2-fixing mode yield as well or better than optimally-fertilized plants under western Canadian prairie conditions (Rennie, 1985).

Legumes derive approximately one-half of their N requirements from N_2 fixation; the remainder comes from the mineral (fertilizer + soil) N pools (Table 2). The main benefit of N_2 fixation is a substitution of fertilizer by atmospheric N. Soil N is still used and must be replenished in the subsequent cropping year.

Table 2. Maximum N_2 fixation in western Canadian legumes as determined by ^{15}N isotope dilution (Rennie, 1985).

Legume	Cultivar	% Ndfa†	N_2 Fixed	Reference
Glycine max (soybean)	Chippewa	23-58	10-92	Rennie et al. 1978
	X005	67	115	Rennie et al. 1982
	Various USA	50	100	Ham & Caldwell 1978
Phaseolus vulgaris (field bean)	Various	38-68	40-125	Rennie & Kemp 1983a
	Various USA	30-50	89-90	Westermane al. 1981
	Cascade	56	95	Witty, 1983
Lens culinaris (lentil)	Eston	79-86	188-192	Rennie & Dubetz 1986
	Laird	72-86	129-162	Rennie & Dubetz 1986 Bremer et al. 1988
Pisum sativum (field pea)	Trapper	75-80	169-189	Rennie & Dubetz 1985
	Century	77-82	166-178	Rennie & Dubetz 1985 Bremer et al. 1988
Vicia faba (faba bean)	Various	73-92	138-237	Rennie & Dubetz 1986
	Minden UK	64	174	Witty 1983
Medicago sativum (alfalfa)	Various USA	43	138-224	Heichel et al. 1983

† % Ndfa = % plant N derived from the atmosphere

Table 3. Grain yield (kg ha^{-1}) and ^{15}N—determined N_2 fixation (% Ndfa) of lentils as affected by inoculation with *Rhizobium leguminosarum* strain 99A1 and LiphaTech-C (Bremer et al., 1989).
(Yields averaged over P rates and lentil cultivars)

Rhizobial Strain	Foam Lake		Semans		Kindersley	
	Grain YD	% Ndfa	Grain YD	% Ndfa	Grain YD	% Ndfa
Uninoc	1338a	6.4a	1045a	9.7a	394a	0a
99A1	2268b	48.0c	1418b	28.8c	501a	7.2b
Lipha-C	2048b	31.7b	1203ab	19.6b	441a	2.7a

Rhizobia are known to survive well in many soils. The apparent lack of success of realizing a yield benefit to inoculation of soybeans in the USA has dissuaded Canadian researchers and farmers from the need for inoculation of annual grain legumes such as lentil, pea or field bean in western Canada. Legume inoculants were commonly referred to as "crop insurance". Accordingly, the perceived benefits of inoculants (yield increase, amount of N_2 fixed etc.) are not widely appreciated and inoculation is not always performed properly.

Lentil Inoculants

In 1984, Esso, the University of Saskatchewan, and the Agriculture Development Fund (Saskatchewan Agriculture) jointly funded a project whose goal was to improve soil quality by studying methods of continuous cropping in Saskatchewan. An important component of this study was the evaluation of strains of *Rhizobium leguminosarum* for lentil grown in rotation with wheat. The studies were incorporated into the University of Saskatchewan/ADF "Innovative Acres" Programme designed to evaluate scientific theories in farm-scale field experiments (Saskatchewan Institute of Pedology, 1984-1989).

Four years of trials in different soil types identified a strain of *Rhizobium leguminosarum* 99A1 for lentil that was demonstrability superior to other rhizobia in N_2 fixation (Bremer et al., 1989, 1990) (Table 3).

Further multisite comparisons of the field performance of the several commercial inoculants available in western Canada showed that strain 99A1 continued to result in superior yields relative to uninoculated treatments (S.I.P., 1989). The entire production package for lentil (i.e. starter N, P requirements for this strain) (Bremer et al., 1988, 1989, 1990) has now been established. It was intriguing that strain 99A1 did not respond to increasing rates of N fertilization (Bremer et al., 1989) whereas other strains did. *Rhizobium leguminosarum* strain 99A1 is the exclusive strain of Esso and is now marketed under the Enfix-L label.

Field Pea Inoculants

Lentils are a relatively new crop for western Canada and accordingly, uninoculated lentil plants usually have few if any nodules (Rennie, 1984, 1986; Rennie et al., 1985; Bremer et al., 1989). Peas have been grown for a longer period and nodulation often occurs even when plants are not inoculated.

The challenge for the pea inoculation industry was to identify superior strains of pea rhizobia, to ensure that proper inoculation techniques were used by farmers, and to understand the edaphic conditions that would best support a yield response due to inoculation.

Large-scale field trials at multiple sites in Saskatchewan and Alberta in 1990 and 1991 showed that when available soil N (0-60 cm) was < 30 kg N ha^{-1}, a significant yield increase due to inoculation, averaging 300-400 kg ha^{-1}, was achieved in the presence of indigenous soil pea rhizobia (Table 4). All uninoculated peas were nodulated .

Field Beans Inoculants

N_2 fixation can be maximized by judicious manipulation of both the plant host and the rhizobial strain because of the known genetic specificity of the symbiosis (Holl, 1983; Phillips, 1991). Strains of rhizobium do not have to be native to our region. In the case of field bean (*Phaseolus vulgaris* L), the best strains for southern Alberta conditions were isolated from acidic soils of Colombia (Rennie and Kemp, 1983a) (Table 5).

Similarly, inoculation with the same rhizobial strain can have dramatically different effects on the yields of different cultivars of a crop such as field beans (Table 6).

The agronomic impact of N_2 fixation associated with legumes is necessarily limited by the hectares cropped to these plant species (Table 7). The western Canadian market is dominated by crops such as wheat, canola and barley and unfortunately 8.6M hectares of summerfallow.

Table 4. Yield response of field pea in Saskatchewan and Alberta to inoculation with *Rhizobium leguminosarum* strain 128C56G (Enfix-P).

	Grain yield (kg ha^{-1}) 1990					
Rhizobial strain	Vauxhall	Picture Butte	Redwater	Outlook	Biggar	Prince Albert
Cultivar:	Radley	Harrier	Radley	Radley	Express	Express
Uninoc	3752	2539	4121	3998	2104	4340
Enfix-P	4141	3155	4354	4341	3020	4960
P<.05	*	*	*	*	*	*
Yield Increase						
kg ha^{-1}	389	616	233	343	916	620
bu ac^{-1}	6	9	3	5	14	9

	1991						
Rhizobial strain	Alvena	Alvena	Smuts	Smuts	Tisdale	Lacombe	Redwater
Cultivar:	Express	Titan	Express	Titan	Radley	Radley	Radley
Uninoc	2607	2155	2465	1031	2551	2544	4200
Enfix-P	3479	2369	3097	2451	3013	3025	4330
P<0.05	*	*	*	*	*	*	*
Yield Increase							
kg/ha	872	214	632	520	462	491	130
bu/ac	13	3	0	9	8	8	2

The economic benefit of these yield increases was dramatic (approximately $25/acres in increased yield based on $4-$5 per bushel of pea) and resulted in a return on investment for using inoculant of >10/1 for the farmer.

Table 5. Effect of strains of *Rhizobium phaseoli* on N_2 fixation of *Phaseolus vulgaris* cv Aurora under southern Alberta conditions (Rennie and Kemp, 1983a).

R. phaseoli strain	Origin	N_2 fixed (^{15}N isotope dilution)
		(kg N ha^{-1})
899	Colombia	119
640	Colombia	113
Lipha Commercial	USA	106
904	Brazil	103
727	USA	75
127	Colombia	52
676	UK	38
57	USA	0

Table 6. Effect of cultivars of *Phaseolus vulgaris* L.(field bean) on N_2 fixation when inocualted with *Rhizobium leguminosarum bv phaseoli* at Vauxhall, AB (Rennie and Kemp, 1983b).

Cultivar	N_2 Fixed
	(kg N ha^{-1})
GN 1140	125
Aurora	93
Kentwood	75
Comtesse de Chambord	56
Redkloud	45
Limelight	50

Table 7. Hectares of legumes and other principle crops planted in western Canada (Rennie, 1991)

Legume	Alberta	Saskatchewan	Manitoba	Total
	---------- planted ha (x 1,000) ----------			
Alfalfa	161	40	40	201
Field bean	10	1	8	19
Lentil	40	177	55	272
Pea	65	78	57	200
		(Millions of Hectares)		
Principle Crops				23.0
Specialty Crops				0.9
Fallow				8.6
Hay				3.7

Table 8. Yield response to inoculation with three competitive inoculants for field pea (1990/1991).

Product	Alvena 1991	Smuts 1991	Vauxhall 1990	Biggar 1990	P. Albert 1990	P. Butte 1990
Cultivar:	Express	Express	Radley	Express	Express	Harrier
Uninoc	2607a	2465a	3824a	2140a	4340a	6105a
Enfix-P	3479b	3097b	3984a	3120b	4960c	6201a
Lipha-C	3539b	2934ab	3808a	2710ab	3810ab	6244a
MBR	ND	2703a	3510b	2670ab	4310ab	ND

It is now clear that under western Canadian agricultural conditions legumes such as field pea, lentil and field bean benefit from rhizobial inoculation, even in the presence of indigenous soil rhizobia.

Comparison of Commercial Legume Inoculant Products

Canadian farmers are beginning to recognize that all legume inoculants are not the same, that factors such as rhizobial strain, quality of the inoculant formulation, effectiveness of the "sticking" agent and general quality control during inoculant manufacture can have a very real impact on the final yield benefit of inoculating legumes.

Field trials in western Canada in 1990 and 1991 showed clearly:

1) The benefit of inoculation relative to uninoculated control, and
2) The differences between three commercially available inoculants for field peas (Table 8). Enfix-P averaged 396 kg ha^{-1}, LiphaTech-C 362 kg ha^{-1} (a non-significant difference) and MicroBioRhizogen (MBR) -138 kg ha^{-1} relative to uninoculated control samples.

Similar differences were documented for legume inoculant products for lentil (Table 9). Based on these 1989 trials across Saskatchewan and the other data presented in this paper, Enfix-L (strain 99A1) averaged yield increases of 216 kg ha^{-1}, LiphaTech-C 116 kg ha^{-1} and MBR 26 kg ha^{-1} compared to the uninoculated control plots. The superiority of *R. leguminosarum* strain 99A1 under western Canadian conditions is obvious.

The key to maximizing N_2 fixation in legumes is to plan for success by:

1) Growing legumes on soils with < 30 kg ha^{-1} available N ha^{-1} (Rennie, 1991).
2) Inoculating with superior N_2 fixing strains (Rennie and Kemp, 1983a; Rennie and Dubetz, 1984a; Bremer et al., 1988; Rice, 1982; Rice and Olsen, 1983).
3) Inoculating using a sticking agent (Elegba and Rennie, 1984) to glue the inoculant to the seed.
4) Avoiding any toxic seed-applied pesticides (Rennie and Dubetz, 1984b; Rennie et al., 1985).

Table 9. Comparison of grain yield benefit of inoculation of lentil with different strains of *Rhizobium leguminosarum*

Rhizobial strain	Kindersley	Outlook	Perdue	Shaunavon	Star City	Zealandia	Average
Uninoc	671	2154	898	801	780	1328	1105
Enfix-L	826	2489	1161	1320	771	1350	1321
Lipha-C	778	2308	1073	1202	738	1225	1221
MBR	661	2150	958	1043	686	1286	1131
Grip	876	2251	1105	1433	593	1174	1239
lsd (P<0.05)	150	176	208	262	NS	NS	180

Table 10. Dinitrogen fixation associated with non-legumes (Rennie and Larson, 1981).

Crop	Common name	Delta ^{15}N	% N_2 fixed*
Agropyron	Pubescent wheatgrass	5.13	0
Elymus angustus	Wild rye	2.31	55
A. elongatum	Tall wheatgrass	3.04	41
A. dasystachyum	Northern wheatgrass	3.00	42
Melilotus officiales	Yellow sweet clover	0.22	100
Medicago sativa	Alfalfa	0.82	84
Astragalus cicer	Cicer milk-vetch	1.90	63
Soil N		7.00	--

*Estimated by ^{15}N isotope dilution using pubsecent wheatgrass as non-fixing control plant

Table 11. Effect of PROVIDE on the yield of wheat (37 location summary) on soils testing low for P (Courtesy of Philom Bios, the Saskatchewan Wheat Pool and Westco).

	Yield			
P_2O_5 Applied	Uninoculated	+ PROVIDE	Yield change	P (<0.05)
----------		(kg ha^{-1})	----------	
0	3009	3074	65	0.01
10	3162	3228	66	0.01
20	3240	3283	43	0.05
30	3310	3294	(16)	0.45

5) Applying starter N and P as required by soil tests (Bremer et al., 1988).

Dinitrogen Fixation-Non-Legumes

Nitrogen balance studies in tropical savannahs (Neyra and Dobereiner, 1977; van Berkum and Bohlool, 1980) and in tropical and temperate grasslands (Rennie and Rennie, 1983) have suggested that crops in these N-starved ecosystems are gaining N from sources other than the mineral N pool. Significant amounts of N_2 fixation have been documented (Table 10) based on the ^{15}N natural abundance technique calculations.

Non-fixing crops, such as Pubescent wheatgrass have a $\delta\ ^{15}N$ similar to that of the soil from which their N requirements were derived. In contrast, non-legumes such as Cicer milk-vetch and Wild rye have $\delta\ ^{15}N$ values suggesting that a significant portion of their N came from N_2 fixation. These $\delta\ ^{15}N$ values are similar to those of the legumes (alfalfa, yellow sweet clover) which derive a majority of their N from the atmosphere.

Wheat

The research history of N_2 fixation associated with western Canada's foremost crop, wheat, is worthy of note. This is an example of meticulous and innovative research conducted by Agriculture Canada, National Research Council and the University of British Columbia over a decade by four individual scientists (Neal and Larson, 1976; Rennie, 1981b; Rennie and Thomas, 1987; Kucey 1988a,b; Chanway et al., 1988a,b).

Neal and Larson (1976) isolated an acetylene-reducing (indicative of N_2 fixation Postgate 1972) *Bacillus spp* associated with the roots of hard red spring wheat on a Lethbridge soil that had been continuously cropped to wheat for 20 years without the addition of fertilizer N. This *Bacillus* strain C-11-25 was shown to fix N_2 and to actively colonize the rhizosphere of wheat (Larson and Neal, 1978). Field trials using ^{15}N isotope dilution proved that up to 10% of the plant's N uptake was derived from N_2 fixation (Rennie 1981b; Rennie and Larson, 1981; Rennie and Rennie, 1983).

Genetic specificity between the host plant cultivar and *Bacillus* and a tropical isolate of *Azospirillum* Sp7 was immediately recognized (Rennie et al., 1983; Rennie and Rennie,

1983; Chanway et al., 1988a,b, 1991). N_2 fixation associated with Canadian HRS wheats was shown to be controlled by chromosome pairs responsible for tolerance of wheat to common root rot, *Cochliobolus sativa* (Rennie and Rennie, 1983). By genetic manipulation of the 5B chromosome in Cadet-Rescue disomic chromosome substitution lines, this associative N_2 fixation could be turned on or turned off (Rennie, 1981b).

The genetic inheritance of the ability of the western Canadian HRS wheat plant to support N_2 fixation was clearly documented (Fig. 1). The ability of the plant to support N_2 fixation was not a dominant trait and had not been used as a basis for selection during the evolution of current HRS varieties (Rennie and Thomas, 1987). Katepwa, which now dominates the HRS market is, fortunately, capable of supporting N_2 fixation (Rennie and Thomas, 1987; Kucey, 1988a,b; Chanway et al., 1988a,b).

Further research showed that these microorganisms exuded plant growth regulating compounds such as cytokinins and indole-3-acetic acid (IAA) (Kucey, 1988a) which actually altered the plant's rooting system (Kucey, 1988b). Fixation of N_2 by the *Bacillus* C-11-25 strain was confirmed (Kucey, 1988a,b) but the main effect of the *Azospirillum sp* was to secrete hormones which altered the rooting system and enhanced nutrient uptake.

It is now clear that certain cultivars of HRS wheat such as Katepwa, but not Neepawa (Rennie and Thomas, 1987; Chanway et al., 1988a,b), do benefit from N_2 fixation associated with it's rooting system. Unfortunately, this research is not continuing.

Nutrient Availability

Phosphorus

Microorganisms are known to exude a wide variety of acids (eg., lactic, oxalic, succinic, citric, gluconic, malonic, tartaric) which will reduce soil pH thus solubilizing calcium phosphates (Kucey et al., 1989).

Brown (1974) reviewed the literature on the ability of microorganisms to solubilize chemically-bound P in soils. *Azotobacter chrococcum* and *Bacillus megaterium var*

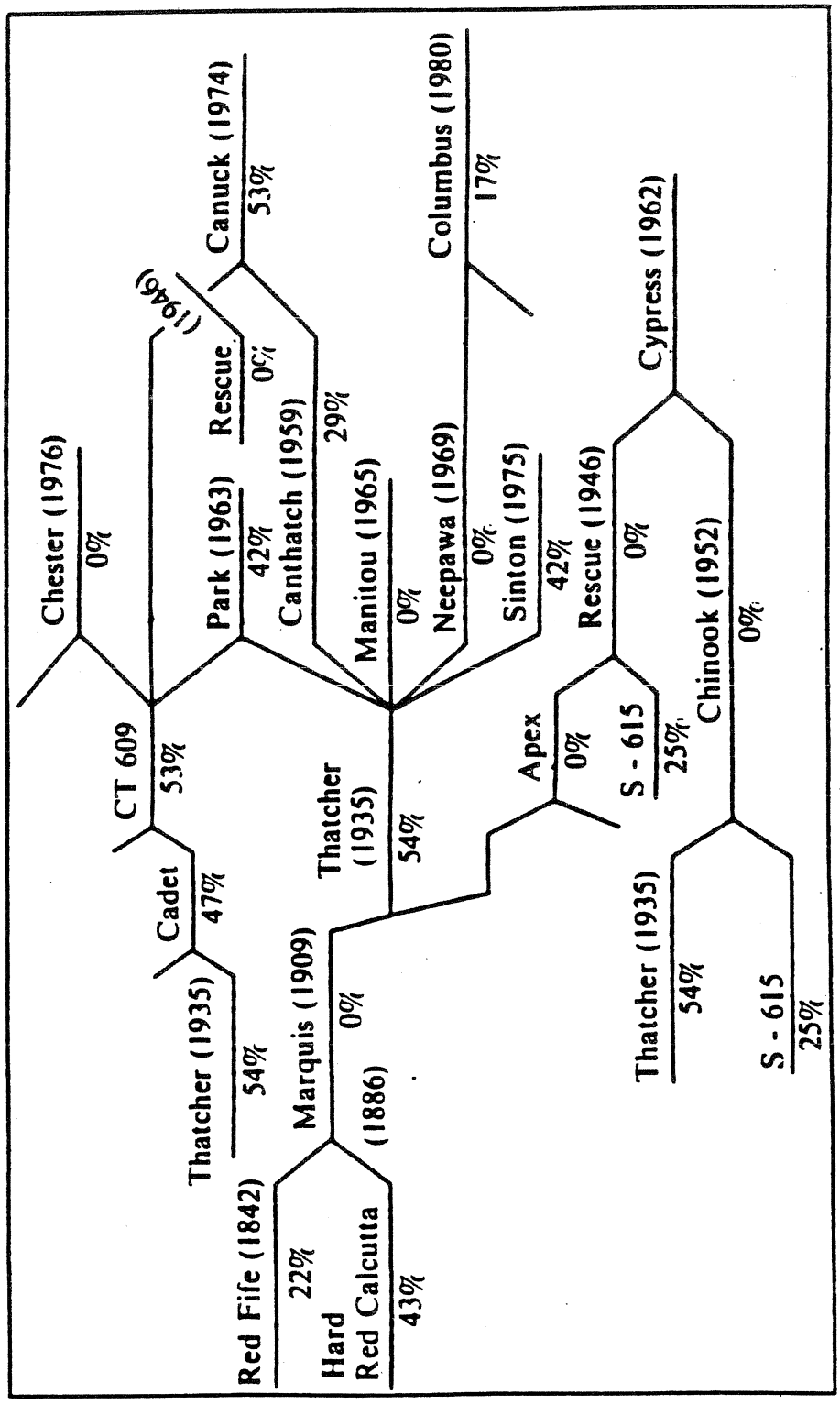

Figure 1. Inheritance of N_2-fixing ability in Canadian spring wheats.

phosphataticum were used for decades as a soil inoculant in the USSR (ca 10 M Ha in 1957) (Mishutin, 1966, 1972) with variable results. These results were never duplicated in other countries (Brown, 1974; Kucey et al., 1989).

Phosphate-solubilizing bacteria and fungi are common in western Canadian soils (0.5 and 0.1%, respectively, on the total bacterial and fungal populations of 29 southern Alberta soils). Most of the fungi are either *Penicillium* or *Aspergillus spp*. Fungi are generally superior to bacteria in their ability to solubilize soil calcium phosphate or rock phosphates and retain this ability through successive sub-culturing (Kucey, 1983). Yields of wheat (Kucey, 1987) and canola (Kucey and Leggett, 1989) fertilized with rock phosphate and inoculated with the fungus *Penicillium bilajii* (designated as PB-50) were similar to those receiving equivalent levels of P_2O_5 as monammonium phosphate in a Vauxhall soil (Orthic Brown chernozem). The beneficial effect of PB-50 could be further enhanced when the pots were inoculated with VAM (Kucey et al., 1989).

Based on these studies, *Penicillium bilajii* was licensed by Agriculture Canada to Philom Bios for commercial development. Further product development and evaluation undertaken jointly by Philom Bios, the Saskatchewan Wheat Pool and Dow-Elanco led to registration under the Fertilizers Act of the first biological plant inoculant in Canada as PB-50 in 1990. PB-50 was marketed by Dow-Elanco as PROVIDE in 1991.

Results indicated small but significant P uptake increases for wheat (Fig. 2) on low to medium phosphate soils, i.e., at sites where a response to fertilizer P addition was anticipated (Gleddie et al., 1991).

On soils with higher soil phosphate levels (>20 kg ha^{-1} available P), there was no response to inoculation with PROVIDE (Table 12) (Gleddie et al., 1991).

Research by the University of Manitoba further supports the claim that the observed P uptake increases with wheat (Table 11) are largely due to enhanced uptake of P in the earlier growth stages (Fig. 2).

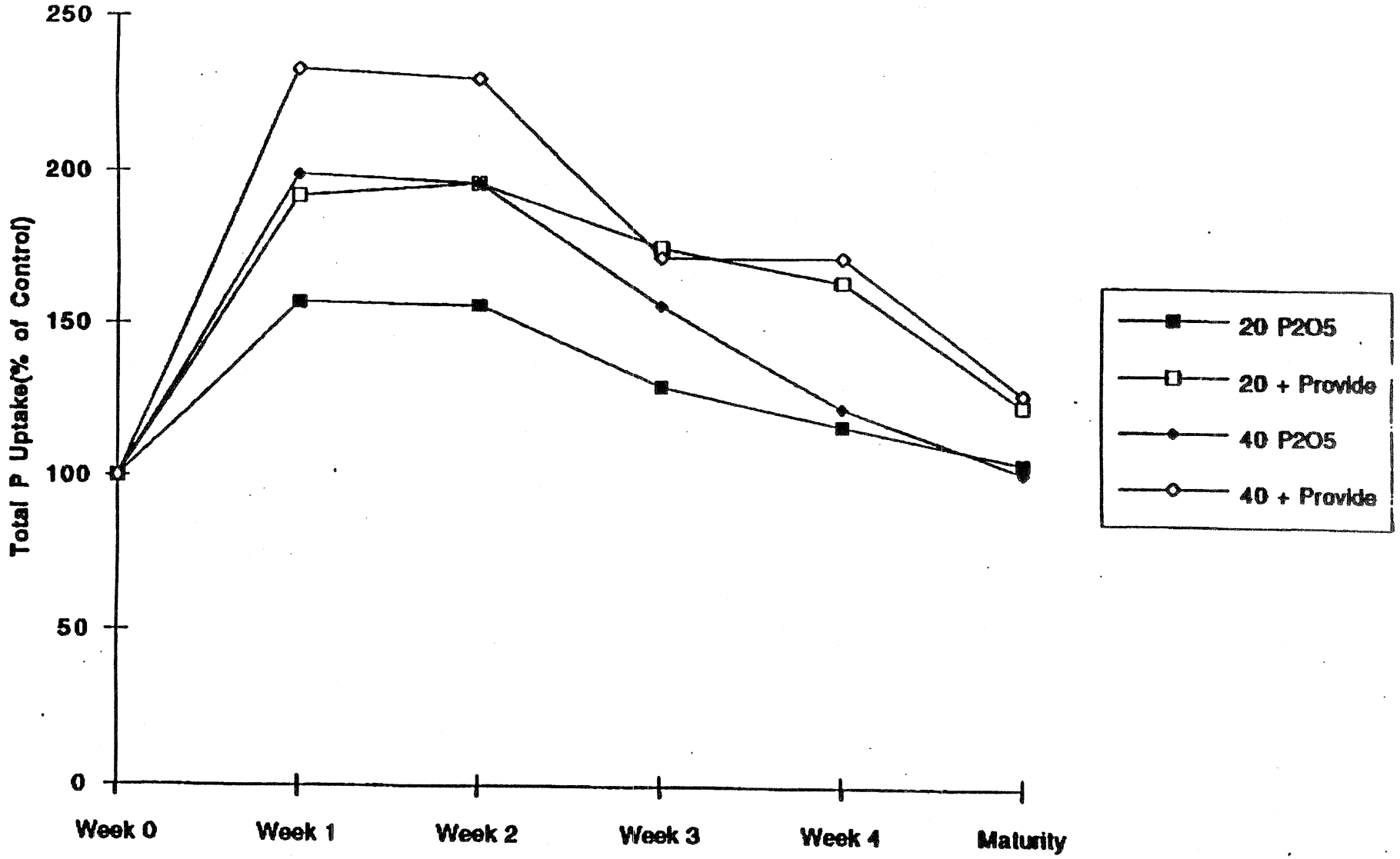

Figure 2. Phosphorus uptake at 20 and 40 kg P_2O_5 ha^{-1} as affected by inoculation with PB-50 (courtesy of the Saskatchewan Wheat Pool); summary of six field trials in 1989 and 1990 conducted by the University of Manitoba.

Table 12. Effect of PROVIDE on the yield of wheat (18 location summary) on soils testing adequate for P (data courtesy of Philom Bios, the Saskatchewan Wheat Pool and Westco).

P_2O_5 Applied	Uninoculated	Yield + PROVIDE	Yield Change	P (<0.05)
		(kg ha^{-1})		
0	3427	3425	(2)	0.94
10	3460	3478	18	0.57
20	3486	3471	(15)	0.65
30	3487	3517	30	0.35

Table 13. Stimulation of nodulation in legumes by nodule-promoting rhizobacteria.

Crop	PGPR microorganisms	Reference
Alfalfa	*Azospirillum brasilense* Cd	Yaholom et al. (1987)
Soybean	*Pseudomonas fluorescens* SSJ2	Nishykima et al. (1988)
	Pseudomonas spp, Serratia sp	Fuhrman & Wollum (1989)
	Bacillus cereus UW85	Halverson&Handelsman (1991)
	P. fluoresecens & putida	Polonenko et al. (1987)
Lentil/Pea	*Pseudomonas spp.*	Chanway et al. (1989)

Table 14. Examples of microbials that are available commercially for biological control of plant diseases (Reddy, 1991).

Antagonist	Disease	Pathogen	Crop
Agrobacterium strain 84	Crown gall	*A. tumefaciens*	Horticulture
Peniophora giganteea	Butt rot	*H. annosum*	Conifers
Trichoderma spp	Damping-off	*Phythium*	
Bacillus subtilis A13	Seedling diseases	Many pathogens	Peanuts
Pseudomonas fluoresens	Seedling diseases	Many pathogens	Cotton

PROVIDE's claim to enhance the uptake of fertilizer P by stimulating greater root development appears valid at field sites where a response to traditional P fertilization can be anticipated.

Phosphorus-VAM (vesicular-arbuscular mycorrhizae)

^{32}P experiments (Asea et al., 1988) show that mycorrhizal and non VAM plants utilize the same sources of soil P. VAM do not utilize unavailable P sources, they simply utilize available solution forms more efficiently (Kucey et al., 1989).

The major action of mycorrhizal fungi is to facilitate plant P uptake by increasing the absorptive surface area of the root and to extend the P depletion zone away from the root surface (Hall, 1988; Lindermann, 1988; Kucey et al., 1989).

Uptake of P by maize in Ontario is markedly increased in undisturbed soils, presumably due to the presence of undisrupted VAM mycelium (Evans and Miller, 1990). Disturbing soils by tillage decreased the ability of VAM to colonize maize roots (Fairchild and Miller, 1990).

Unfortunately, it is not possible to reproduce VAM independently of the plant host and this severely limits our ability to directly manipulate and thus enhance its effect in the field. Ontario studies (McGonible et al., 1990) have successfully manipulated VAM *in situ* by adopting conservation tillage practices in Ontario. Tillage disrupted the VAM (Evans and Miller, 1990; Fairchild and Miller, 1990) sheering its hyphae and thus destroying its ability to function properly. However this reduction in P-supplying power of the VAM-maize root association may not be agronomically important if soil P concentrations are adequate (Miller, personal communication).

The readers are referred to Chapter 4 for details on the apparent higher P fertility levels of stubble than fallow (tillage). One of the reasons for the lower frequency of response to P fertilization in stubble soils is their measured higher VAM populations.

Sulphur

Lower consumption of coal-based fossil fuels and higher environmental standards are reducing the deposition of atmospheric S onto agricultural soils. This has resulted in a need for S fertilization particularly for high S-requiring crops such as alfalfa and canola. To meet this S demand, ammonium sulphate fertilizer use has increased in western Canada.

Interest in the utilization of microorganisms to enhance the availability of fertilizer or soil S has waned because of the ease of application and nutrient availability of new granular ammonium sulphate fertilizers. Numerous microorganisms are known to oxidize elemental S to plant-available sulphate but most past research has concentrated on the autotrophic S-oxidizing microbial population. Research in Saskatchewan (Gupta et al., 1988; Lawrence et al., 1988; Lawrence and Germida, 1988a,b) showed that heterotrophs rather than autotrophs dominate typical soils. The percentage of microorganisms capable of oxidizing S was greater in the rhizosphere than the non-rhizosphere but this varied somewhat depending on the crop grown and the soil type. The rate at which elemental S could be oxidized was dependent on the soil biomass; thiosulphate producers outnumbered sulphate producers by 1000-fold.

The practical application of utilizing this knowledge and of inoculating elemental S with S-oxidizing microorganisms was unfortunately severely limited by:

1) Lack of understanding of the ecology of the key microorganisms involved.
2) The need for finely ground S as a substrate. This product is highly explosive.

PLANT GROWTH REGULATORS

Theory

The theoretical ability of microorganisms to affect plant growth is clear.

*Plant growth is regulated by plant growth regulators (PGRs).

*Microorganisms exude PGRs (Tien et al., 1979; Okon and Hardar, 1987; Kucey, 1988a).

The challenge is to combine these two facts into an agronomically useful product. The distinct advantage of inoculating plants with PGPRs compared to using chemical PGRs is that the plant, by means of its communication with the microorganisms, can dictate the timing and concentration of PGR required.

An excellent example exists in the legume-rhizobium symbiosis. Rhizobia are attracted to their host plant by immunological recognition signals, lectins (Bohlool and Schmidt, 1974). Alfalfa rhizobia do not infect lentil or pea and visa versa. Legumes exude tryptophan which is metabolized into indol acetic acid (IAA). This IAA causes root hair curling which is the direct precursor to the first invasion steps of the rhizobium into the plant root for the process of nodulation (Dart, 1977). The entire rhizobial infection process is controlled by the genetics of both the rhizobia and the host legume and is very complex (Dart, 1977; Rennie, 1981c; Holl, 1983; Phillips, 1991).

The proposed role of PGPRs is to stimulate more plant root development. This in turn should ensure that the plant "sees" more nutrients (soil and fertilizer) and moisture, two factors which severely limit crop yields in western Canada.

Under the semi-arid conditions that prevail in western Canada, it has been clearly demonstrated that any factor which stimulates root development (i.e., optimal fertility) may increase water use efficiency substantially. PGPRs that enhance root development and activity would play a similar role.

PGPR Enhancement of Nutrient Uptake

Bacterial inoculation (primarily with N_2-fixing bacteria) has resulted in significant yield increases under field conditions for wheat, maize, sorghum and potato These yield increases occur most frequently when moderate fertilizer N rates are applied or in soils of intermediate fertility levels suggesting that N_2 fixation is not the sole stimulatory mechanism (Okon and Hardar, 1987).

The mechanism(s) of this bacterial stimulation are not completely understood but several possibilities have been identified:

1) *Alteration of rooting volumes:* bacterially-produced auxins (IAA), gibberellins and cytokinins cause secondary root proliferation and thus increase effective rooting volumes. This permits more efficient and extensive exploration of mineral pools (Tien et al., 1979; Barea and Brown, 1974; Jain and Patriquin, 1984; Kucey, 1988a). Other bacteria restrict rooting zones vertically but not horizontally. This has implications for more efficient fertilizer placement (Kucey, 1988b).

   Kucey (1988b) in an elegant series of experiments using ^{32}P-labelling techniques, documented that *Azospirillum Cd*, a Brazilian isolate shown by Rennie (1983) and Kucey (1988a) not to fix N_2 with most Canadian wheat cultivars, did indeed alter and sometimes restrict the rooting pattern of inoculated wheat. In contrast, the *Bacillus* C-11-25 shown by Rennie and Kucey to fix up to 10% of the plant's N requirement, did not alter the plant's rooting pattern (Kucey, 1988b).

2) *Cell Wall Degradation:* PGPRs produce polygalacturonic acid transeliminase (PATE) which may soften root cell walls thus enhancing nutrient uptake (Okon and Harder, 1987).

3) *Ethylene Production:* PGPRs produce C_2H_4 under microaerophilic conditions which restricts root growth (Lynch, 1987, 1988)

4) *Siderophore Production:* PGPRs produce siderophores which complex or chelate soil Fe making it more plant-available and less available to root pathogens (Kloepper et al., 1989).

5) *Phosphorus Availability:* PGPRs secrete acids and chelating compounds which increase P availability in calcareous soils (Kucey various)

6) *Disease Suppression:* PGPRs alter the rhizosphere of potato suppressing the competitive ability of the pathogen *Erwinia spp* (Kloepper et al., 1989);

7) *Altered cell-wall permeability:* Okon and Harder, 1987. showed PGPRs could alter cell wall permeability.

8) *Emergence Promotion:* PGPRs may hormonally stimulate seedling emergence resulting in more uniform and earlier stands

Nodulation Promotion

If PGPRs have the ability to stimulate root development in cereals, the same effect could be observable in legumes (Table 13).

PGPRs have been implicated in the stimulation of numbers of nodules (Polonenko et al., 1987; Chanway et al., 1989), number of sites for nodulation (Yaholom et al., 1987), competitive ability of different rhizobial strains to compete for nodule occupancy (Fuhrman and Wollum, 1989) and suppression of root diseases harmful to nodulation (Halverson and Handelsman, 1991). Nodule-promoting rhizobacteria (NPRs)have been shown to stimulate yield in the Peace cultivar of alfalfa (Hynes et al., 1991) near Beaverlodge and in pea and lentil cultivars in Saskatchewan and Alberta (Rennie et al., 1992).

BIOLOGICAL CONTROL OF SOIL-BORNE DISEASES

The ecological line between pathogenic and beneficial soil bacteria is fine indeed. The Family *Rhizobacaeae* is defined as microorganisms which cause cortical hypertrophies on plant roots (Buchman and Gibbons, 1974). *Rhizobaceae* have only two genera, *Rhizobacaeae* which form beneficial N_2-fixing nodules in the legume symbiosis and *Agrobactereaceae* which form crown galls on crop roots. In fact, the pathogenicity of agrobacterium is used to insert beneficial genes (such as the Bt toxin) into crops to make them resistant to insect diseases.

Many bacteria have demonstrated the capability of controlling soil-borne plant pathogens (Weller, 1988; Reddy, 1991). The prime example is *Agrobacterium radiobacteria* strain 84 which controls crown gall caused by *A. tumefaciens* and is sold

commercially in Australia and the USA. *Bacillus subtilis* A13 which is an inhibitor of several plant pathogens, is sold for treatment of peanut under the name QUANTUM-4000 (Gustafson Seeds). DAGGER is sold by Ecogen for control of cotton soil-borne root pathogens.

The proposed and documented mechanisms of biocontrol activity (BCA) are many (Weller, 1988; Lynch, 1988; Reddy, 1991) and include:

1) Substrate competition (BCAs deplete the pathogens food supplies).
2) Niche exclusion (BCAs bind to the root surface effectively occupying all potential pathogen binding sites).
3) Siderophores (low molecular weight, high affinity Fe^3 chelators that transport Fe into bacterial cells). Siderophores produced by pseudemonads deplete the limited Fe^3 supply in soils and limit its availability to pathogens and suppress their growth (Bakker et al., 1988).
4) Antibiotics: *Bacillus polymyxa* produces polymyxin B, a gram-negative antibiotic. Most rhizosphere bacteria are gram negative.
5) Induced Resistance: Bacteria produce phytolexins which induce resistance in white bean infected with *Fusarium* sp., the equivalent of "immunization.".
6) Parasitism: *Trichoderma* parasitizes some *Phythium spp.*

COMMERCIAL PRODUCTS

Rhizobial inoculants are the best example of commercially available PGPRs in western Canada. Several rhizobial strains have been registered as amendments under the Fertilizers Act (Table 15).

Table 15 indicates that a majority of the legume inoculants sold in western Canada have been scientifically evaluated under western Canadian conditions.

PB-50, the P-solubilizing fungus developed by Agriculture Canada and commercialized by Philom Bios is the first and only PGPR to be registered under the

Table 15. Rhizobial inoculants registered for use in Canada supported by on-site field evaluations.

Crop	Rhizobial	Company	Reference
Alfalfa	NRG 185	LiphaTech	Rice (1982)
	NRG 185	MicroBioRhizogen	Rice (1982)
	Balsac	LiphaTech	Bordeleau (pers. comm.)
	NGA-3	LiphaTech	Smith (pers. comm.)
Lentil	99A1	Esso	Rennie(1991)
	ICAR 20 (RGL14)	MicroBioRhiozgen	S.I.P. (1987)
		LiphaTech	
Pea	128C56G	Esso	Unpublished
	RPG4	MicroBioRhizogen	Innov. Acres (87)
	128C56G	LiphaTech	
Soybean	523c	LiphaTech	Hume (pers. comm.)
	523c	AgriGenetics	
Fieldbean	RGB22	MicroBioRhizogen	Stephens (pers.comm.)

amended Fertilizers Act. Two other agricultural biotechnology companies (Esso-Ag Biologicals, MicroBioRhizogen) are actively pursuing product development in the PGPR and BCA area but the technical challenges are great and the regulatory process is not well defined.

None of the biocontrol products mentioned above (Table 8) are currently marketed in western Canada.

THE FUTURE

Manipulating the Rhizosphere

There are many challenges to manipulating the rhizosphere. The following are the most realistic and are presented in order of most imminent impact but also increasing technology challenge:

N_2-fixing Rhizobium for legumes

The legume-rhizobium symbiosis is the best example of successful manipulation of the rhizosphere. It is clearly possible in western Canada to enhance legume crop yields by inoculating seeds with microorganisms but only if:

1) Rhizobium strain is highly competitive in nodule formation and highly efficient in fixing N_2.
2) Soil conditions favour rhizobial survival, nodulation and N_2 fixation.
3) Inoculant is properly prepared, stored and applied.

Thus, by "planning for success", the producer can be assured of excellent legume yields with little or no fertilizer N application if he applies good rhizobial inoculants (Rennie, 1991). Planning for success entails the following:

The Soil:

*< 20-30 kg N ha^{-1} available in the spring to 12 cm.

*Few or no indigenous rhizobia

*Agronomic conditions that favour a healthy crop (plant survival is much more critical than rhizobial survival)

*Appropriate soil nutrition (starter N as required, suitable P, S, Fe, Mn nutrition)

The Bacterium:

*Highly infective (i.e. forms nodules) and effective (i.e., fixes N_2)

*Selected for host plant specificity (right strain for each cultivar)

The Inoculant:

*Minimum of 10^9 cfu g^{-1} or -mL^{-1} of inoculant

*Stored under cool conditions

*Inoculant is physically "fixed" to the seed such that the rhizobia will be physically adjacent to the potential nodulation sites on the growing root tip. (P fertilization is an excellent analogy here).

VAM for P uptake

It is not possible to directly manipulate VAM in the rhizosphere for western Canadian field crops because:

1) VAM cannot be reproduced as an inoculant independent of the host plant, ie. bioengineering limits commercial potential.
2) VAM is indigenous to all western Canadian crop species except brassicas which exude some toxin which precludes VAM survival in the brassica rhizosphere.

The influence of VAM, particularly on P nutrition and moisture availability of the plant can be dramatically and positively affected by agronomic practices which ensure the physical integrity of the VAM hyphal system, i.e.

1) Minimum or zero tillage.
2) Maintenance of crop residues because of their effect on soil aeration, moisture etc.

BCAs for control of soil-borne crop diseases

Biological control has potential for control of root and seedling diseases for:

Pre- and post-emergence damping off of cereals and oilseeds

Brown spot of wheat

Black leg of canola

Common root rot of cereals and oilseeds

The success of biocontrol will depend on:

1) Consistency of pathogen control
2) The development of technology to ensure proper inoculation(pre-inoculation preferred)

The future impact of biocontrol on western Canadian agriculture will further depend on the success of research presently underway and planned in the following areas:

1) Technology successes (see above)
2) Environmental sensitivities to seed-applied pesticides, i.e., will the public be willing to accept the introduction of non-indigenous microorganisms into the ecosystem.

3) Relative cost/benefit in product performance and pricing of biocontrol relative to traditional chemical control.

PGPRs for cereals and legumes

PGPRs do stimulate yields of cereals and oilseeds under western Canadian conditions by:

1) N_2 fixation (up to 10% of the plant's N requirement)
2) Enhanced nutrient and moisture uptake due to increasing rooting volumes or nutrient transport permeability
3) Hormonal stimulation of seed emergence

A target of 10% increase in seed yield is readily and reproducibly achievable.

Adoption of this technology, as with BCAs will be determined by:

1) Environmental and regulatory issues for biotechnology
2) Market pricing of nutrient inputs relative to seed yields(i.e. cost/benefit of PGPRs)
3) Performance comparisons between traditional nutrient sources and PGPRs.

ROADBLOCKS TO PROGRESS

The major challenges are:

(1) Consistency of PGPR performance:
(2) Mode of Action
(3) Inoculation Technology
(4) Cost Benefit

PGPRs either work well or do not work and in both instances, the reasons are not clear. The key is a better understanding of the mode of action of PGPRs so that planning for success will be possible. Assuming that inoculation technology is satisfactory (i.e., that PGPRs are a fully embodied technology), PGPRs will have an impact only if product performance returns a consistent and economically meaningful benefit to the farmer. This

is not a problem with rhizobial inoculants but the progress of PGPR research for wheat will suffer because of current low wheat prices.

The major technical road block is a proper inoculation formulation and inoculant system amenable to the large areas of cereals in western Canada. It is currently difficult to convince farmers to inoculate the relatively small area of legumes in western Canada. What will happen with quarter sections of wheat? This is an engineering challenge which will soon be overcome.

However, the ability to consistently ensure survival of the PGPR in the rhizosphere, and to compete with indigenous microorganisms, will be difficult.

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