Laboratory to the Marketplace: Scientific Challenges in Commercializing a Phosphate Solubilizing Microorganism

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Abstract
The commercialization of phosphate inoculant is a challenging process. The active ingredient of the phosphate inoculant ***JumpStart***® (*P. bilaiae)* was isolated in 1982. Although the concept of P solubilization was proven, much additional research was required. Cost effective manufacturing processes, packaging and QA systems, and easy-to-use, shelf stable formulations needed to be developed. Extensive field research to confirm efficacy was needed. Comprehensive data on compatibility with seed-applied pesticides were required. Development continues to be an on-going process with the use of the product on new crops, improved production methods and formulations, new applications, and continuing market research to monitor changing farmer needs.

Key words: inoculant commercialization, JumpStart, *Penicillium bilaiae*, phosphate solubilization.

Introduction

Commercializing any product is a difficult process. Commercializing a biologically based product presents additional challenges, and commercializing a biological product aimed at enhancing phosphorus nutrition is even more difficult. This paper describes the scientific challenges involved in commercializing and marketing ***JumpStart***® (a phosphate inoculant based on the phosphate solubilizing fungus *Penicillium bilaiae).*

Kucey (1983) isolated *Penicillium bilaiae* from Canadian prairie soils in 1983. He demonstrated that the organism could solubilize phosphate (P) on agar plates and in liquid culture (Kucey 1983). He also demonstrated that inoculating soil with the fungus could increase the growth and P uptake in wheat and beans in greenhouse and field trials. Philom Bios acquired the rights to commercialize this product in 1986. Agriculture and Agri-Food Canada (AAFC) obtained a patent (Canadian patents 1,308,270 and 1,308,566) and a royalty agreement was signed between Philom Bios and AAFC. This agreement was crafted such that the royalty funds would go back into research rather than into general government funds. Philom Bios has paid AAFC $2.0 million from 1990 to 2003.

The concept of *P. bilaiae* as a phosphate inoculant had been demonstrated (Kucey 1983, Kucey 1987, Kucey 1988, Asea *et al.* 1988). However, a tremendous amount of work was required to

make this into a commercial product. Extensive field trials were conducted to prove that the organism was effective over a wide range of soil, environmental conditions, and crop types. These data supported the registration of the product. We had to develop a production method, a commercially acceptable formulation, a quality assurance process, and information on application strategies. Initial knowledge of the behavior and mode of action was limited.

The phosphate inoculant was registered for use on wheat in 1990 and is now registered on most major crops in Western Canada. From a small number of hectares inoculated in the first year, 1.9 million hectares were inoculated in 2003. These advances have resulted from an increasing commitment to research and a constant willingness to develop cooperative projects with researchers across Canada.

Field Research

Inoculant organisms are affected by many soil and environmental factors and must be thoroughly tested under conditions that will exist where they will be used.

Pre-commercialization

Field trials had been done on two crops (wheat and beans) at one site (Kucey 1988). The inoculated bran media had been applied in furrow (by hand) in these studies.

Commercialization

Philom Bios did not have the resources to carry out all the field tests needed for registration. Philom Bios joined with Dow Elanco Canada (DEC) to carry out field tests across the Canadian Prairies. The Saskatchewan Wheat Pool (SWP) also conducted independent trials to ensure an unbiased source on information for the registration package. As an in-furrow application using bran material was impractical for large scale farming operations, a seed treatment was used. Thirty-eight trials were established across the three Canadian Prairie Provinces (Manitoba, Saskatchewan and Alberta) in 1987 and 1988. All trials were arranged in a split plot experimental design. The control (untreated) and *P. bilaiae* treatments were compared over four rates of P_2O_5 (0, 10, 20, 30 g P_2O_5 / ha). *P. bilaiae* increased yield and P uptake in wheat (Table 1) at the lower rates of P application.

Subsequent field work proved the fungus could also increase early season biomass (dry matter) in canola, pulses, and alfalfa (Figure 1). Determining the effect of *P. bilaiae* on pulses necessitated the examination of the interaction of the fungus with rhizobium inoculants. We found that the addition of *P. bilaiae* further increased yield gains achieved with the introduction of rhizobia alone. While some of the yield gains may have been due to increases in phosphorus uptake, additional benefits may have resulted from the effect of the fungus on root growth. Recent work at the University of Manitoba has shown that root growth (Vessey and Heisinger 2000) and root hair development are increased by *P. bilaiae* (Gulden and Vessey 2000). *P.bilaiae* may increase the absorptive capacity of roots, which may lead to increased uptake of P and other nutrients, or even water uptake (Vessey and Heisinger 2000). The increased root hair development could also increase the ability of rhizobial inoculants to nodulate the host plant, as rhizobia enter the root through the root hairs. An increased understanding of the effects of *P. bilaiae* on host plant development will help us refine our recommendations on the use of ***JumpStart***®.

On-Going
The field program continues to increase as the market expands and sales move into the northern U.S. states. In addition, as each new crop or new formulation is added, field trials are conducted to ensure inoculant effectiveness.

Registration
Inoculants in Canada are registered as fertilizer supplements under the Fertilizers Act administered by the Canadian Food Inspection Agency (CFIA). This process requires proof that the organism is safe and that it will perform according to the claim on the label. All new inoculants must go through this process and any claim on the label (including pesticide compatibility) must be reviewed by the CFIA.

Pre-Commercialization
The product could not be marketed and sold until it was registered. The product was first registered for use on wheat in 1990 under the name ***PB50***®. Philom Bios, DEC, and SWP conducted field studies to gather data to support registration. The field work monitored P uptake as well as yield (Gleddie, *et al.* 1991) to show that the fungus increased the phosphate nutrition of the plants. Chambers and Yeomans (1991) monitored P uptake in wheat from emergence to maturity at four phosphate levels (0, 10, 20, and 40 kg P_2O_5 kg / ha). P uptake was increased at all levels of P but the greatest effect was seen at 10 and 20 kg P_2O_5/ ha. This information was backed up by greenhouse experiments with ^{32}P using wheat and flax (Chambers and Yeomans 1991). They found that plants inoculated with *P. bilaiae* increased tissue P concentration, primarily through increased soil P contributions (as opposed to fertilizer P).

Commercialization
Every new crop must be registered and so field and compatibility data were submitted and reviewed before canola, pea, lentil, and alfalfa were added to the label in 1992, 1993, 1993, and 1996, respectively. The amount of data Philom Bios submits to the CFIA is increasing rapidly as new crops and chemical compatibility information are added to the label and new formulations are developed. This is beginning to present a problem as the large volume of material that has to be reviewed creates a backlog in the system and delays the introduction of new applications. The market introduction of a granular formulation was delayed by one year due to delays in the registration system.

On Going
Philom Bios continues to work with the CFIA to try to streamline the registration process. We ensure that they approve of the format of our reports and the statistical analysis we use before we send in large submissions.

Production and Formulation
A good quality inoculant must be able to survive storage, desiccation after inoculation onto the seed, and natural competition in the rhizosphere (Maurise *et al.* 2001). Formulation development is a complex process and is still more of an art than a science (Daigle and Connick 1990). Production and formulation research must be done with constant consideration for the costs involved in the process. Many production methods or formulation ingredients are far too costly

to be used in a product designed for prairie agriculture. Losses in recovery and processing and allowances for maintaining stability during storage and use can double the cost of the active ingredient. Formulation additives, packaging, the cost of the quality assurance program, and shipping costs, can add another increment so that the cost of the final product may be up to three times higher than the original active ingredient.

Researchers must consider costs throughout the entire commercial process before making decisions that will set production methods. The theoretical example shown in Table 2 illustrates this point. The researcher has grown the organism in two different media, Tryptic Soy Broth (TSB) and Sucrose Yeast Extract (SYE). The yield is 10 fold higher in the TSB than in SYE. Unfortunately, TSB costs 38 times as much as SYE, which makes it four times more expensive "per cell". This information alone would lead a researcher to reject the TSB. The cost of the media, however, is not always the determining factor in the cost of an entire production campaign. The cost of operating a large-scale fermentation may exceed the cost of growth media to such a degree that reducing the number of fermentation batches can markedly reduce the cost of the active ingredient. In the example shown in Table 2, using TSB can reduce the number of fermentation batches from ten to one. This reduces the production cost by $437,000 despite the extra media costs incurred by using TSB.

Pre-Commercialization

Kucey (1988) used a straw substrate to produce spores for greenhouse and field trials. This was effective for the small trials but impractical on a commercial scale. The production process was cumbersome and only a limited amount of material could be produced. Twenty-three pyrex dishes were needed to produce the inoculum for five research sites (approximately 0.1 hectares). The material was applied by hand as it could not be applied through a commercial seeder.

Commercialization

A liquid fermentation method was developed which produced sufficient spores in one batch to inoculate 25,000 hectares of wheat. The spores were collected and processed into a dry powder, which had to be kept frozen to maintain viability (effectiveness). This frozen powder, ***PB50***®, was introduced to the market place in 1990. The economics were prohibitive however, as up to 80 % of the viability was lost during the drying process. The development of a frozen liquid formulation, ***Provide***®, solved this problem and raised the number of hectares treated to 126,000 per fermentation batch. This material, however, was still inconvenient to use and store as the product had to be kept frozen in a non frost-free freezer. The development of ***JumpStart***®. a room temperature stable wettable powder addressed this issue. The stability of a formulation on seed is also an important criterion when assessing the acceptability of a formulation. The move from the ***Provide***® frozen liquid formulation to the dry room temperature stable ***JumpStart***® wettable powder increased the half life of the fungus on seed from 10 to 35 days.

Ongoing Research

The search for improved formulations is ongoing. The current formulation has a half –life of 4 weeks at 28^{o}C. In the Canadian prairies the product will rarely be subjected to long periods above 25^{o}C, so this is an acceptable shelf-life. As we move into warmer climates, increased stability at higher temperatures is a commercial imperative.

Quality Assurance
Once an inoculant is developed for any crop, or area, there must be strict adherence to quality standards (Hedge *et al.* 1999). Substandard materials restrict the popularity and acceptability of the product (Hedge *et al.* 1999).

Pre-Commercialization
Before commercialization, the *P. bilaiae* spores had been produced and applied with the rate based on the amount of dry material added per meter of furrow (Kucey 1983). This did not allow for the development of a quality control procedure as the quality and the amount of fungus in a gram of substrate varied from batch to batch.

Commercialization
The first step in the development of a quality assurance program was to set parameters for the product. In Canada, rhizobium products are regulated by the Fertilizers Act and the number of bacteria per seed is predetermined based on seed size (Olsen *et al.* 1994). As the phosphate inoculant was a new product, Philom Bios assisted Ottawa in establishing a standard (the minimum number of spores per seed required for efficacy) by submitting field data. Once this level was determined, a quality assurance system could be developed. As the quality (cfu per g) varies from batch to batch, each batch must be evaluated separately. Enough samples must be taken from each batch to ensure that a statistically valid number could be obtained. We chose a dilution plating method and cfu count as the basis for our quality assurance system because a large number of samples could be assayed without expensive analytical tools. This assay is, however, a tedious process that becomes more cumbersome as production volume increases. The number of agar plates for the quality assurance program for the phosphate inoculant at Philom Bios has increased from 3,000 in 1992 to 80,000 in 2003.

On-Going
The increase in production is increasing our need to develop a fast, reliable method to assess the number of viable spores in our product to replace the cfu plate assay.

Application
The inoculant must conform to standard application practices used on-farm. An inoculant can be applied either as a seed treatment or as in-furrow application. Seed inoculation is the most commonly used method of application. Seed applied pesticides are also commonly used and many seed treatment chemicals contain fungicides that reduce the survival of *P. bilaiae* on seed. We therefore must be concerned about the ability of the inoculant organism to survive on fungicide treated seed long enough to be effective in the field.

Pre-commercialization
Fungicide use on wheat was not universally practiced in the 1980's. All of the early field trials were conducted with untreated seed.

Commercialization
The use of seed treatment fungicides increased in the 1990s. Farmers needed these materials to protect their crop from increased disease pressure and could not omit the fungicide in order to use the phosphate inoculant. We therefor developed a system to test the compatibility of *P.*

bilaiae with commonly used seed applied chemicals. The chemical and the inoculant are applied to seed and the population of the *P. bilaiae* on the seed is determined. The seed is stored at room temperature and the population of *P. bilaiae* is monitored for up to four weeks. The application methods used to apply the two materials mimic the application methods a farmer would use. The fungicide and the inoculant may be mixed together in a slurry (tank-mix), applied to seed at the same time through separate hoses (simultaneous), or the chemical may be applied to the seed first and allowed to dry before the inoculant is added (sequential). Although the tank-mix is usually the most damaging to the fungus, farmers prefer this method, as it is quick and easy (Table 3).

Generally, the planting window is the longest when the two materials are applied sequentially (Table 3). Each chemical formulation must be analyzed separately as it is often the formulation ingredients, rather than the active ingredient that affects the fungus. A change in the formulation of either the chemical or the inoculant will potentially alter the planting window.

On Going

We must continue to test our materials with new chemicals and formulations. Our current compatibility tests look at loss in viability on seed due to chemicals but do not look directly at efficacy. We plan to add a greenhouse or field-screening component to these tests. Seed coating companies are constantly looking at polymers to improve seed flow in seeders, protect rhizobial inoculants from environmental stresses, and manipulate seed germination. We constantly evaluate these materials to determine if they will reduce or increase the survival of the *P. bilaiae* on seed.

Conclusion

The development of a commercial phosphate inoculant has been a challenging and rewarding process. The procedures had to be developed as we went as there was no "user's manual" to lead us through the process. The challenges do not end with the commercialization process but continue to arise as we improve our product and processes, keep pace with new developments in agriculture, and expand our market.

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Figure 1. Effect of *P. bilaiae* on early season biomass.

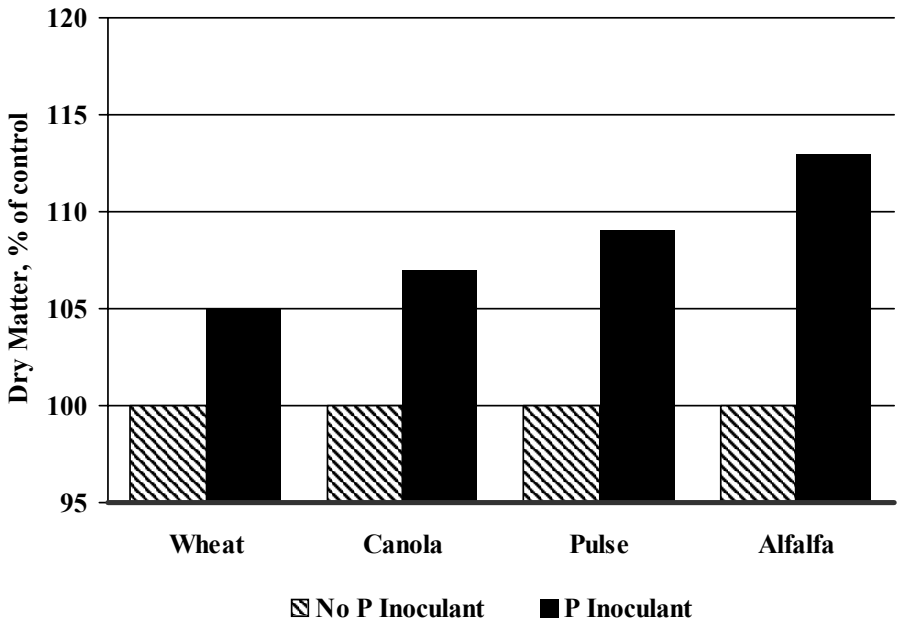

Table 1. Effect of *P. bilaiae* on the yield of wheat. Multiple year field summary of 38 locations

	Mean Yield (Kg/ha)			
Phosphate Applied	Untreated	*P. bilaiae*	Difference	Statistical significance
0	2771	2827	56	0.01
10	2909	2958	49	0.04
20	2930	2962	32	0.15
30	2962	2948	(14)	0.54

From Hnatowich *et al.* 1990

Table 3. Planting windows for use of ***JumpStart***®

with commonly used fungicides on wheat.

	Planting Window (days)		
Seed Treatment*	TankMix	Simultaneous	Sequential
Bare Seed	15	15	15
Baytan® 30	10	10	10
DB Green	do not use	2	7
Proseed®	do not use	10	10
Vitavax® Single	do not use	10	10
Vitaflo® 280	do not use	1	4

÷ Baytan 30, Vitaflo 280 Vitavax Single Solution, are registered trademarks of Uniroyal Chemical Ltd.

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