Canadian Biopesticides and Bioherbicides

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Abstract

Biopesticide technology is emerging as a viable and environmentally-friendly pest management tool in agriculture. Although the current global biopesticide market is small in comparison to the synthetic pesticide market, biopesticides are expected to exceed $1 billion in annual sales. The Canadian public's demand for safer foods and concern for the environment have encouraged initiatives to develop alternatives to conventional pesticides. Biopesticides are classed by Health Canada as reduced risk products that are less hazardous to human health and the environment and they represent the next generation of pest control products with novel modes of action. Agriculture and Agri-Food Canada (AAFC) has invested in a strategic priority to promote the development and commercialization of this technology. This paper presents a summary of new and emerging Canadian biopesticides and bioherbicides being developed by AAFC researchers.

Introduction

Biopesticides are composed of naturally-occurring microorganisms that kill, suppress, or reduce the vigor of the target pest, whether it is a plant disease, insect pest, or weed (Bailey et al., 2010; Boyetchko, 2005; Boyetchko et al., 2002). These microorganisms are often responsible for significant reductions in pest populations under natural conditions, but artificial inoculations of crop pests is a more common method of employing these microbials as biopesticides. The biopesticide active ingredient is generally a bacterial, fungal, or viral propagule and a major feature of biopesticides is that they are mass-produced through fermentation technology, formulated and applied at high inoculum rates to the pest, and somewhat analogous to the application of chemical pesticides. They are also generally regarded as safe due to their lower human and mammalian toxicity and are not expected to survive and persist in the environment. Historically, biopesticides were often selected as host-specific pest control agents to a particular species of pest, but it is currently recognized that broad-spectrum activity against a wide range of pest species is preferred to increase the economic and market potential of the biopesticide product. Many of our modern biopesticides are being commercialized for broad-spectrum use.

The Global Pesticide and Biopesticide Markets

Synthetic pesticide sales in 2006 globally were valued at $31.2 billion, down by 12% from the previous five years. This declining trend is expected to continue largely due to increased health hazards associated with many conventional pesticides. Moreover, many commonly used chemical pesticides are under regulatory review by the Pest Management Regulatory Agency (PMRA) with Health Canada (Bailey et al., 2010). In addition, 60 chemical pesticides used in Canadian agriculture have been banned by OECD countries. The global biopesticide market is valued at US$350-400 million or 1.5 - 2.5% of the total pesticide products, with *Bacillus thuringiensis* (*Bt*) dominating the market at >90%. The registration of microbials and their incorporation into pest management strategies is increasing worldwide due to their potential as relatively specific and environmentally safe pest control options accepted by both conventional and organic producers. The demand for biopesticides is rapidly increasing and sales are expected to exceed $1 billion by 2010 (Bailey et al., 2010). This market will continue to be spurred by the application of integrated pest management practices and expansion of organic farming worldwide. In Canada, prior to 1990, only 18 microbial biopesticides were registered by PMRA, but since then, an additional 65 new biopesticides (new actives and/or new formulations) were registered. Reports indicate that a total of 32 novel microbial active ingredients were registered in Canada as of 2010; 12 belonging to different bacterial species, 11 to fungi, 6 to nematodes, 2 to viruses and 1 to protozoans (Kabaluk et al., 2010).

Rationale for Biopesticides

A number of factors that have precipitated the need to develop biopesticides as alternatives to synthetic pesticides can be divided into three main categories: government legislation, public perception, and industry drivers (Bailey et al., 2010; Boyetchko, 2005; Boyetchko and Svircev, 2009). In Canada, new legislation in over 73 municipalities banned the use of synthetic pesticides for cosmetic use within their city limits. In April, 2009, Ontario legislated a province-wide ban of chemicals in urban environments. Hence, this created an ideal opportunity for the development of biopesticides as green alternatives. Despite the removal of these products from within urban areas, few options were available to city managers and home gardeners to replace these pest control products. However, the de-registration or phasing out of several older pesticides and the policy of PMRA to support the registration of reduced risk pest control products that have lower mammalian toxicity encourages the development and adoption of biopesticides for the Canadian consumer.

Impetus for biopesticides is the result of an increased awareness by the public (i.e. the general public and agricultural producers/farmers) of environmental issues related to spray drift and pesticide residues in the soil, water, and food (Boyetchko, 2005). The public's desire for environmentally friendly alternatives to chemical pesticides and food safety were cited as major concerns by consumers in California (Bruhn et al., 1992) and Canadians revealed a strong preference for pesticide-free foods (Magnusson and Cranfield, 2005, Thakore, 2006). Moreover, public perception of continued use of chemical pesticides in agriculture was determined to be detrimental to soil and water quality and elimination of chemical residues in the food supply was considered to be a more favourable option (Bailey et al., 2010). These issues are driving the need for a paradigm shift in the manner in which our food supply is managed. Biopesticide use alleviates the heavy dependency on chemical pesticides while meeting the needs of organic and pesticide-free crop production. Management of pest populations that have developed resistance to chemicals can be achieved through the application of biopesticides that exhibit novel modes of action, thereby mitigating or delaying the development of pest populations showing single or multiple resistance to specific chemical fungicides, herbicides, and insecticides.

Many of the microbial active ingredients have been discovered and evaluated in either government research laboratories or universities (Bailey et al., 2010; Boyetchko, 2005). Commercialization of the majority of biopesticide products has been pursued by small to medium sized companies rather than by large multi-national businesses. For example, out of 150 biopesticide products being developed by Canadian companies, 100 were being developed by small companies of less than 50 employees, 40 by medium-sized companies comprised of 50-149 employees, and only 10 by companies with more than 149 employees (Bailey et al., 2010). However, the smaller companies often lack the infrastructure and capital to conduct early discoveries or invest in research until it is at the later stages of the innovation chain and closer to registration. Multi-national agriculture companies diverted much of their R&D efforts from discovery of new chemistries and have not made significant investments in biopesticide product development due to market size limitations. In fact, there have been fewer new chemistries discovered for pesticides in recent years. In addition, these large firms have invested in infrastructure devoted more to discovery of synthetically-derived compounds whereas it would take additional investment in infrastructure and expertise to alter their discovery programs towards microbial-based technology. Investment in microbial fermentation is required and formulation of chemical pesticides is not always compatible with microbial-based pesticides. Although multi-national companies often possess large infrastructure and devote resources towards R&D, these companies have been investing in seed technologies which involve GM crops, with the major crops such as corn, soybean, rice, potato, and cotton being the main focus. These companies are not necessarily addressing all the needs of other markets such as pulse crops, small and large fruit crops and high value field and greenhouse vegetable crops. This therefore provides an opportunity to smaller industry to fill this niche.

Strategic Framework for Biopesticide Development

The cost and consistency of the microbial organisms with varying environmental conditions, efficient mass production (i.e. fermentation), formulation and delivery systems, and prolonged shelf-life have always been a challenge to the development and commercialization of biopesticide products (Boyetchko, 2005; Boyetchko and Peng, 2004; Hynes and Boyetchko, 2006). Past experiences have shown extensive complexities within pest/pathogen systems, which affected product development. Research must tackle these complexities with more innovative approaches by addressing biological, environmental, and technological issues to achieve success (Boyetchko and Rosskopf, 2006; Boyetchko et al., 2002).

Contrary to popular belief by many government and university researchers that once a microbial organism has been discovered in the laboratory, the final biopesticide product is imminent, a biopesticide product is one where all the platform technologies involved in product development are considered (Figure 1) (Boyetchko and Peng, 2004). The taxonomy, biological characterization, mode of action, and efficacy are among the factors that are core to the selection of the biopesticide organism, but the platform technologies including fermentation, formulation, and application/delivery systems are integral to the actual "product" itself. In fact, discovery of a promising organism as the active ingredient is often the easiest part of the battle, but it is the selection and development of the appropriate fermentation system (e.g. liquid/submerged vs. solid-state) for economic scale-up in combination with the most suitable formulation (e.g. liquid/spray application vs. granule/seed treatment/soil application) and the type of application method (e.g. foliar- vs. soil- applied) that determine the biopesticide product and its performance (Boyetchko and Rosskopf, 2006; Hynes and Boyetchko, 2006). Indeed, "orphaned" biopesticide technologies have been the result of difficulties in selecting the right fermentation and/or formulation process during the product development phase as these processes will determine whether the product is a go or no-go for industry. Moreover, the fermentation process can affect shelf life and stability in the formulation phase while formulation ingredients and down-stream processing will influence delivery and application. Conversely, the type of application method required will often dictate the most appropriate formulation required to achieve effective pest control under field conditions. These three platform technologies are often inter-related and any minor changes to each of these processes will have impact on each other, thus potentially leading to significant improvements in biopesticide performance in the field.

The Process of Biopesticide Development

Biopesticide discovery and development follows a process of incremental steps that are unique for each target pest-biopesticide system. The Biopesticide Innovation Chain (Figure 2) was a concept developed that depicts several critical stages for developing a biopesticide product using a series of "Go vs No-Go" criteria in order to make decisions on the feasibility of the organism and target (Boyetchko and Svircev, 2009; Svircev et al., 2010a). This concept is applicable to any type of biopesticide (i.e. bacteria, fungi, viruses, natural product) and is appropriate to any type of crop pest (i.e. weed, invertebrate/insect pest, plant pathogen).

The early stages link discovery to proof-of-concept and platform technology development, as described earlier. Basic assessments of biology, environment, biochemistry, and small-scale fermentation and formulation are conducted under laboratory, greenhouse and field conditions, with emphasis placed on characterization, safety, and practicality of the biopesticide organism (Bailey et al., 2010; Boyetchko and Svircev, 2009; Svircev et al., 2010). The importance of regulatory and market considerations cannot be underestimated since they will dictate the success or failure for commercialization (Bailey et al., 2010). Although these two areas are not necessarily a research consideration to scientists, they are equally important because defining the field of use and the market will pre-determine the experimentation and scientific data required to register the product. Another key feature of the innovation chain is that it encourages development of novel technology platforms that can be expanded to other potential applications and crop pests, whether this is for multiple use patterns, application methods, broadening host targets, new production systems, or be integrated into other crop production systems (Boyetchko and Rosskopf, 2006; Hynes and Boyetchko 2006). Although a great deal of science and understanding of the microbial physiology and biochemistry are involved, there is as much art required in this aspect of product development.

The later stages in the innovation chain (i.e. application development and technology transfer) test the robustness of the earlier decisions by actively working with various stakeholders involved in biopesticide development. These include the industry partner, other collaborators, and regulators to develop the data required for the registration package. Moving into commercial scale-up can still affect the final stages of product development, often requiring significant investment back into technology development to move from the bench to pilot scale to commercial manufacture. Commercial scale-up, registration, and technology adoption is usually directed by an industry partner, but the champion of the project is usually the lead scientist or inventor who should work collaboratively on the project with industry until technology adoption has occurred. The corporate memory or "know-how" and intuition of the scientist, along with the scientific expertise, should not be overlooked by the industry partner. Past experience shows that it can ease the transition phases and add potential new product value. The successful development of a biopesticide is a combination of science, art, entrepreneurship, and serendipity which can take over10-15 years to complete.

Examples of Biopesticides in various stages of R&D

Scientists at Agriculture and Agri-Food Canada (AAFC) have assembled a team of researchers from across the country working on different crop pests. The innovation chain shows the similar components and common linkages within microbial biopesticide research. As a group, we are able to bring a diversity of biopesticide candidates into discovery and proof-of-concept phases using similar processes and shared facilities. Past experience has shown us that some of the gaps identified in biopesticide research, including fermentation and formulation technology, has led to "orphaned" biopesticides due to lack of critical mass of expertise in the different areas. As a multidisciplinary team, we are able to capitalize on the collective knowledge, skill sets, and expertise. We've also targeted economically important crop pests identified by stakeholders (industry, grower/producer groups) in order to advance several promising biopesticide projects.

A. Biopesticides for Plant Diseases:

Post-harvest diseases of fruit crops

Brown rot disease caused by *Monilinia fructicola* is the most serious disease of stone fruits, including peach (Zhou and Sholberg, 2002a) and can affect more than half the crop before harvest, with the remaining crop being very susceptible to post-harvest decay particularly during storage and transit. Similarly, *Penicillium expansum*, and *Botrytis cinerea*, which cause blue mold and grey mold, respectively, in a wide range of greenhouse and field crops, can cause significant fruit rot (Zhou and Sholberg, 2002b). Several microbial isolates obtained from food products purchased from local food stores or from various commercial retailers as culture starters for wine, beer, cheese and yogurt were used in an *in vivo* screening protocol to determine their ability to control *M. fructicola* on peach (Zhou et al., 2008). In co-inoculation tests of wounded fruits with eight microbial isolates and the pathogen, a *Bacillus* sp. C06 was capable of reducing disease incidence by 92%. Additional efficacy tests where conducted where the peach fruit was immersed in a suspension of *M. fructicola* spores containing either whole culture or cells only. While the fruit inoculated with the pathogen alone had 93% incidence of the disease, application of the bacterial cultures of *Bacillus* C06 led to 100% disease control. Peach decay was also significantly reduced by this microbial isolate. Similar results were obtained using *Pseudomonas syringae* isolates against blue mold and gray mold on apple (Zhou et al., 2001). Further taxonomic studies revealed that C06 is *B. amyloliquefaciens* and detailed studies are ongoing to identify the active ingredient(s) secreted by bacterial that promote the biopesticidal activity against this disease.

Fire Blight Disease in the Orchard

Fire blight disease, caused by *Erwinia amylovora*, in pear and apple causes significant yield loss in commercial fruit orchards (Svircev et al., 2011). Streptomycin has been used to control this disease but resistance to this antibiotic has been reported worldwide. Researchers at AAFC and Brock University have developed a novel approach towards the control of fire blight in the orchard. The system relies on using *Pantoea agglomerans* and bacteriophages, microorganisms commonly found in the orchard ecosystem, to control the fire blight pathogen (Svircev et al., 2010a, 2010b; Svircev et al., 2011). Bacteriophages (or simply "phages") are bacterial viruses that infect specific host bacteria, replicate inside it, and then kill the host cell to release the new phages. *Pantoea agglomerans* has a dual role in this system, acting as a biological control agent and as a carrier for the phages. The carrier permits the continuous production of fresh, infective phages on the flower surface, while competing with the pathogen for the ecological niche provided by the blossom (Lehman et al., 2008). Early field trials in pear and apple orchards have demonstrated that the phage-carrier system can reduce the incidence of diseased blossom clusters by 50%. Research is continuing to identify isolates with high field efficacy, determine the mechanisms of development of phage resistance in host bacterium, develop large scale processing of phage/carrier and to follow the environmental fate of the phages in the orchard ecosystem. The ultimate goal is to develop a biocontrol system that will have efficacy for disease control in the orchard comparable to streptomycin, the industry standard.

Sclerotinia stem rot of canola

Sclerotinia stem rot of canola caused by *Sclerotinia* sclerotiorum causes several million dollars in yield losses annually. This pathogen is also known to have a wide host range, infecting more than 400 plant species. Exploration for bacterial strains as biopesticides against this disease in canola has been ongoing at AAFC for several years. Several bacterial strains of *Pseudomonas* spp. were shown to have inhibitory activity to *Sclerotinia sclerotiorum* (causal agent of sclerotinia stem rot in canola) using a variety of bioassays (Behrouzin et al., 2004). Radial growth rate of mycelia was suppressed by the 8 bacterial strains that showed antifungal activity; 3 of these strains significantly delayed but did not completely inhibit mycelial growth. In addition, 7 of the 8 bacterial strains completely inhibited sclerotial formation. Further, all 10 bacterial strains either completely inhibit or reduce ascospore germination; some of the strains also reduce ascospore viability. In addition, a bacterial strain, *Bacillus subtilis* LEV-006, was found to have broad-spectrum activity against 4 canola pathogens including *S. sclerotiorum, Alternaria brassicae, Leptosphaeria maculans*, and *Rhizoctonia solani* (Hou et al., 2006). The antifungal activities were associated with a low molecular weight peptide complex consisting mostly of the cyclic lipopeptide fengycin A and B, as well as two proteins of 20 and 55 kDa. The 55-kDa protein was similar to vegetative catalase 1, but when expressed in *E. coli*, it did not exhibit the antifungal activity. The 20-kDa antifungal protein was found to be unique and sequences of several peptides were obtained.

B. Biopesticides for Insect pests:

Bacillus thuringiensis (Bt) for control of invertebrate pests

The first and most successful biopesticide used around the world is *Bacillus thuringiensis* (*Bt*) (Côté, 2007). This gram-positive bacterium is characterized by possessing a parasporal inclusion body, called the crystal, which is comprised of proteins that express insecticidal properties against invertebrate species such as lepidopteran, dipteran, and coleopteran pests. There are more than 60,000 strains of *Bt* belonging to more than 82 serovars worldwide. While the majority of these biopesticides exhibit insecticidal activity, activity against nematodes and protozoans have also been documented. At AAFC in St-Jean-sur-Richelieu, scientists have surveyed and characterized novel *Bt* strains for biological control of the tarnished plant bug and free-living nematode (Bélair and Côté, 2004; Wellman-Desbiens, and Côté, 2004). During the process of developing new *Bt* strains on these novel target insect pests, new liquid fermentation media and seven new formulations for various end uses were developed (Côté, 2007). These formulations were developed in collaboration with a small company, AEF Global, Inc. under the Bioprotec™ formulation series and possess different potencies and uses on tree fruits, forestry, household use, and agriculture in either aqueous or dry flowable powder form.

Biological Control of Wireworm using *Metarhizium anisopliae*

Wireworms are a significant insect pest to various economically important crops such as corn, potato, and sugar beet worldwide. Although this pest has traditionally been controlled through the use of synthetic pesticides, a fungal biopesticide, *Metarhizium anisopliae* has been investigated as a reduced-risk pest control product in organic crop production and to address issues related to environmental health. Kabaluk et al. (2007) showed that *M. anisopliae* applied either as a granule, soil incorporation or seed treatment caused significant mortality of wireworm and was attributed to increased mycosis in the field. When living wireworms were retrieved from the soil, following field application of *Metarhizium*, latent infection and proliferation of the biopesticide was observed. A specific strain of the biopesticide, F52, which infects and kills wireworms in the field, was shown to control the insect pest and result in improved stand density and increased foliar fresh weight in corn (Kabaluk and Ericsson, 2007a). In addition, environmental conditions such as soil temperature were found to have an effect on the efficacy of *Metarhizium* (Kabaluk and Ericsson, 2007b). Fatal infection of wireworm was demonstrated when soil temperatures were at least 18°C and when the insect was exposed to *M. anisopliae* for at least 48 hours. Other factors that affect the performance of *Metarhizium* in the field include conidial concentration and food availability. Detailed studies to understand biotic and abiotic factors to optimize biopesticide performance are underway

<u>Cabbage looper/baculoviruses</u>
A serious insect pest in vegetable production in greenhouse is the cabbage looper which causes significant damage to cucumber, tomato, and sweet pepper. Although *Bacillus thuringiensis* (*Bt*) has been used to control this insect pest, resistance to *Bt* has been reported. Erlandson et al. (2007) have been investigating the use of baculoviruses as biologically-based viral insecticides. These baculoviruses, once ingested by the invertebrate pest, can cause mortality within several days, resulting in a cadaver filled with viral occlusion bodies. A highly virulent and infectious isolate of baculovirus, AcMNPV, has demonstrated the ability to control cabbage looper in cucumber production within 5 days post-inoculation, resulting in significant increases in cucumber yield and reductions in fruit damage. Research is being pursued to mass-produce and formulate the baculovirus, with the ultimate goal of bringing the biopesticide to commercial production.

C. Biopesticides for Weeds:

<u>Biological Control of Broadleaved weeds in turf using *Phoma macrostoma*</u>
A fungal pathogen, first discovered and isolated from necrotic foliar tissue on Canada thistle, is being developed as a bioherbicide for control of dandelion and other broadleaved weeds in turf (Graupner et al., 2003; Zhou et al., 2004). The fungus, *Phoma macrostoma*, produces photobleaching compounds called macrocidins which can be applied as either a pre- or post-emergent application in a granular formulation. A solid-state fermentation system is used to mass produce the fungus followed by milling the infected grain containing mycelial fragments, extruding and spheronizing into small flowable granules.

Pseudomonas fluorescens for control of annual grass weeds

A soil-applied bacterium, *Pseudomonas fluorescens* strain BRG100, is being developed as a bioherbicide to control annual grass weeds, green foxtail and wild oat. Boyetchko, 1997; Daigle et al., 2002; Pedras et al., 2003). These naturally-occurring bacteria isolated from prairie soils have exhibited greater than 80% weed control in the laboratory and field. Strain BRG100 produces deleterious bioherbicidal compounds called pseudophomins that either inhibit weed seed germination or suppress root growth. The grass weeds are not affected if the bacteria are applied as a foliar application at the 1-2 leaf stage or greater, and thus must be formulated and applied to the field crop at seeding as a pre-emergent bioherbicide. A novel granule formulation, called pesta, was developed to stabilize and delivery *P. fluorescens* (Daigle et al., 2002). It is manufactured by growing the bacteria in liquid fermentation medium, adding to a solid matrix or dough containing oat flour, extruding into pasta-like noodles, and spheronizing into uniform granules (Hynes and Boyetchko, 2010). The addition of pea starch is used to improve the dispersion characteristics and the granules are dried to a water activity of 0.3 a_w to stabilize the bacterial populations. Using this formulation, a shelf life of 16 months at 8.5 $\log_{10}$ colony-forming units per gram of formulation has been achieved.

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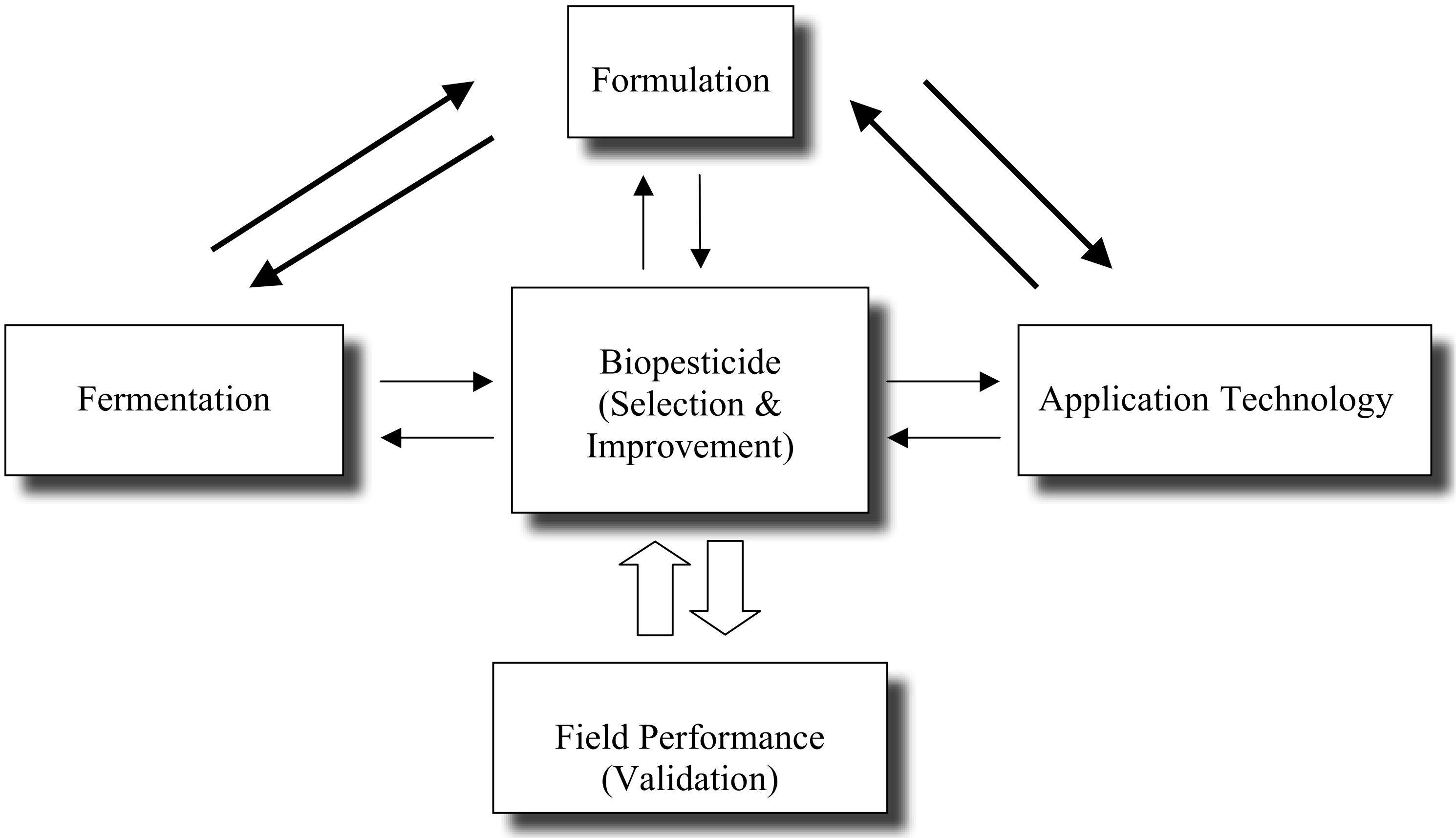
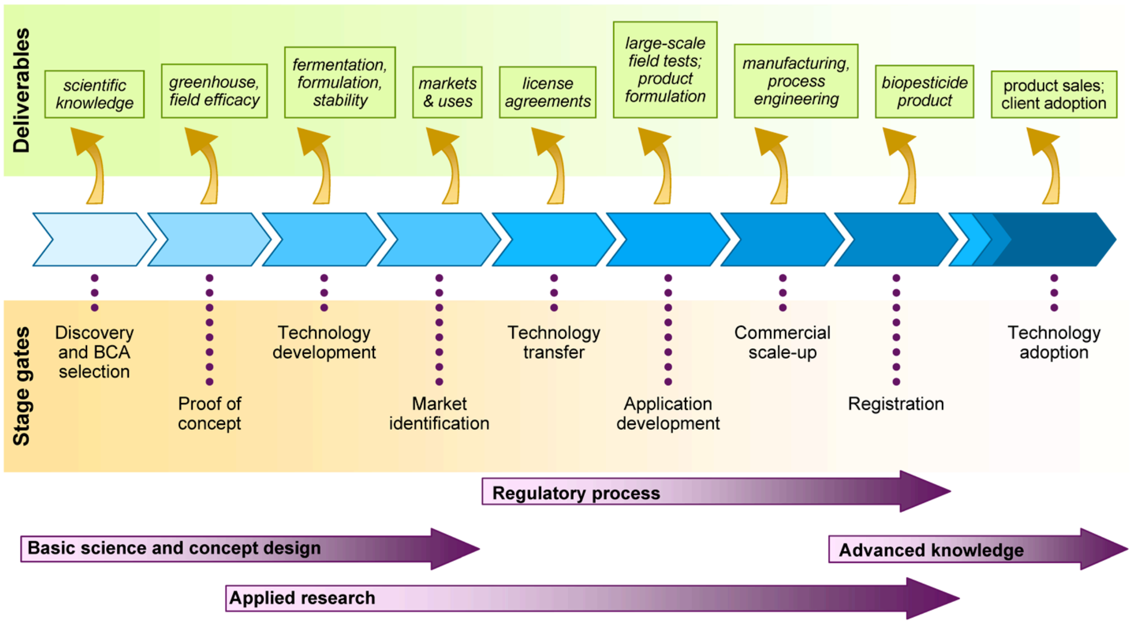


Figure 1. Strategic Framework for Evaluation and Development of Biopesticides

(adapted from Boyetchko and Peng, 2004)

Figure 2. ***A Solution for Delivery of Biopesticides - AAFC Biopesticide Science Innovation Chain***



(adapted from Svircev et al., 2010)